ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

CHANGES IN POLYPHENOLS AND ANTIOXIDANT ACTIVITY DURING THE PROCESSING OF POMEGRANATE INTO NECTAR

M.Sc. THESIS

Ece SÜREK

Department of Food Engineering

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Thesis Advisor: Assist. Prof. Dilara Nilüfer ERDİL

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

NARIN NEKTARA İŞLENMESİNDE POLİFENOLLERDE VE ANTİOKSİDAN AKTİVİTEDEKİ DEĞİŞİMLER

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Ece SÜREK, a **M.Sc.** student of ITU **Graduate School of Science Engineering and Technology** student ID 506101505, successfully defended the **thesis** entitled "**CHANGES IN POLYPHENOLS AND ANTIOXIDANT ACTIVITY DURING THE PROCESSING OF POMEGRANATE INTO NECTAR**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To my mother, my father and my brother

FOREWORD

This master thesis was performed from September 2011 to May 2012 in the Food Engineering Department of Istanbul Technical University. I gratefully acknowledge the financial support for this project from the EU 7th Framework Project ATHENA (Anthocyanin and Polyphenol Bioactives for Health Enhancement through Nutritional Advancement) and AROMA Fruit Juices and Food Industry Inc. Karaman Facility for giving the chance to carry out such a comprehensive project including all the pomegranate concentrate and nectar processing steps, with waste products and products obtained from two different production batches.
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ABBREVIATIONS

ABTS : 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid
ANOVA : Analysis of Variance
CE : Catechin Equivalent
CL : Clarification
CO : Cooling
CON : Concentrate
CUPRAC : Cupric Reducing Antioxidant Capacity
Cyn 3-O-glu : Cyanidin 3-O-glucoside
Cyn 3,5-dOg : Cyanidin 3,5-di-O-glucoside
Del 3-O-glu : Delphinidin 3-O-glucoside
Del 3,5-dOg : Delphinidin 3,5-di-O-glucoside
DPPH : 2,2-diphenyl-1-picrylhydrazyl
DW : Dry Weight
EA : Enzyme Application
FW : Fresh Weight
MEYED : Fruit Juice Industry Association
FRAP : Ferric Reducing Ability of Plasma
GAE : Gallic Acid Equivalent
HCA : Hydroxycinnamic Acid
HBA : Hydroxybenzoic Acid
HDL : High Density Lipoprotein
HPLC : High Performance Liquid Chromatography
HSV : Herpes Simplex Virus
IN : Solution Entering The Dialysis Tubing
IR : Infrared
LC-MS : Liquid Chromatography-Mass Spectrometry
LDL : Low Density Lipoprotein
NE : Nectar
NMR : Nuclear Magnetic Resonance
ORAC : Oxygen Radical Absorbance Capacity

PA : Pomegranate Arils
PAC : Precipitate After Clarification
PAS : Pasteurization
PC : Press Cake
PDA : Photodiode Array Detector
Pel 3-O-glu : Pelargonidin 3-O-glucoside
Pel 3,5-dOg : Pelargonidin 3,5-di-O-glucoside
PG : Postgastric
PM : Mashing
PN : Pasteurized Nectar
PP : Pomegranate Peel
PR : Pressing
PVPP : Polyvinylpolypyrrolidone
RP-HPLC : Reversed Phased High Performance Liquid Chromatography
RSV : Respiratory Syncytial Virus
Rt : Retention Time
RM : Raw Material
QE : Quercetin Equivalent
Q-3-g : Quercetin-3-galactoside
Q-3-BDg : Quercetin-3-β-D-glucoside
OUT : Solution Not Entering The Dialysis Tubing
SD : Standard Deviation
SPSS : Statistical Package for the Social Sciences
TEAC : Trolox Equivalent Antioxidant Capacity
TPTZ : 2,4,6-tripyridyl-s-triazine
Trolox : 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
TSE : Turkish Standards Institution
TURKSTAT : Turkish Statistical Institute
UF : Ultrafiltration
UPLC : Ultra Performance Liquid Chromatography
UV : Ultraviolet

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CHANGES IN POLYPHENOLS AND ANTIOXIDANT ACTIVITY DURING THE PROCESSING OF POMEGRANATE INTO NECTAR

SUMMARY

Pomegranate (*Punica granatum*) is an important fruit due to its high antioxidant potential and phenolic content. The important phenolic compounds in pomegranate are anthocyanins, flavonol glycosides, procyanidins, phenolic acids such as ellagic acid and its derivatives. The reason for its high antioxidant capacity is existence of significant phenolic compounds such as tannins, flavonoids and anthocyanins. Phenolic compounds and polyphenols have important antimutagenic and anticarcinogenic properties due to their antioxidant activity. Moreover, they have protective effects against cardiovascular diseases.

High levels of phenolic compounds available in pomegranate and their high antioxidant activity have increased the interest to pomegranate and its products, especially juices obtained from pomegranate in the last years. There are many research in literature showing the high antioxidant activity of pomegranate. However, phenolic content and antioxidant activity of food depend on several factors such as growth, processing and storage conditions. It is claimed that there can be some changes or losses in phenolic compounds during production of pomegranate juice. Although there are some studies related to changes in phenolic content and antioxidant activity in some processing steps of pomegranate juice production, there has not been any comprehensive research including pomegranate arils, pomegranate peel and all of the processing steps from raw material to the product.

Firstly, the purpose of this study was to investigate changes and losses in total phenolic content, total flavonoid content, total anthocyanin content, total tannin content, total antioxidant activity, polymeric color (%), total antioxidant capacity, major phenolic compounds and anthocyanins during each step of pasteurized pomegranate nectar production (mashing, pressing, cooling, pasteurization, enzyme application, clarification, ultrafiltration, evaporation, nectar, pasteurization of nectar) including whole raw material, pomegranate arils, waste products such as press cake, pomegranate peel, precipitate after clarification. Secondly, for better understanding the real effects on human metabolism, to compare the potential bioavailability of those raw materials, waste products and end products by means of phenolic content, anthocyanin content and antioxidant activity after *in vitro* digestion.

The samples were obtained from industrial scale pomegranate nectar production in Karaman. Samples were collected from two different production in duplicate. They were stored in -80^{o}C in laboratory and then milled by using liquid nitrogen before analysis. Moisture content was measured by vacuum oven method and extraction was performed by using 75% methanol:water solution involving 0.1% formic acid. Standard calibration curves were prepared for each analysis.

Total phenolic content, total tannin content, total anthocyanin content were measured by Folin-Ciocalteu method, vanillin method and pH differential method respectively. Total antioxidant activity was analyzed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, Cupric Reducing Antioxidant Capacity (CUPRAC), Ferric Reducing Ability of Plasma (FRAP) and 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity methods. Polymeric color (%) was measured by using potassium metabisulfite method. Major phenolic compounds (gallic acid, catechin, quercetin-3-β-D-glucoside, quercetin-3-galactoside, ferulic acid, neochlorogenic acid and p-coumaric acid,) and major anthocyanins (cyanidin 3-O-glucoside, cyanidin 3,5-di-O-glucoside, delphinidin 3-O-glucoside, delphinidin 3,5-di-O-glucoside, pelargonidin 3-O-glucoside and pelargonidin 3,5-di-O-glucoside) were determined by using Reversed Phase High Performance Liquid Chromatography coupled with Photodiode Array Detector (RP-HPLC/PDA).

Pomegranate can not be grown at all seasons but it can be consumed for longer durations when it is processed to different products. Due to this fact, bioavailability of pomegranate becomes important and should also be investigated. In this study, not only for the product, arils and raw material, but also for waste products such as peel, press cake and precipitate after clarification, *in vitro* potential bioavailability was evaluated. By using enzymes and *in vitro* digestion method, digestion system in human body was simulated and the effect of processing on bioavailability was investigated. Total antioxidant activity analysis by DPPH method, total phenolic content analysis by Folin-Ciocalteu method, total anthocyanin content analysis by pH differential method and major phenolic compound and anthocyanin analysis by RP-HPLC/PDA were carried out in samples for potential bioavailability evaluation.

All of the data were evaluated statistically using Statistical Package for the Social Sciences (SPSS) program version 16.0. To determine the significant changes between samples one way Analysis of Variance (ANOVA) was applied at at 0.05 significant level followed by Duncan's New Multiple Range Test as post hoc tests. The results were reported as mg equivalents/100 g dry weight (DW). Each analysis was repeated in triplicate for each sample and the results were reported as mean value ± standard deviation.

Generally, pomegranate peel showed higher phenolic content and antioxidant activity than other samples. There was a decrease from raw material to mashing due to losses with pomegranate peel. Pasteurized nectar showed the lowest values and but the effect of pasteurization was not significant in this reduction. According to changes during production from raw material to the product, raw material showed the highest value for total phenolic content, total flavonoid content, total tannin content and all antioxidant activity analysis, 11161.5 mg gallic acid equivalent (GAE)/100g DW, 14127.1 mg quercetin equivalent (QE)/100g DW, 1883.1 mg catechin equivalent (CE)/100g DW and 60677.1 for CUPRAC, 26327.0 for DPPH, 15754.9 for FRAP and 30762.6 mg Trolox equivalent antioxidant capacity (TEAC)/100g DW for ABTS, respectively. Cooling step significantly increased total anthocyanin content value to 162.0 mg cyn-3-gly/100g DW. For all analyses, except for total anthocyanin content, there was a decrease from raw material to mashing. The product had the lowest values, except for total flavonoid content. There was no change for CUPRAC, total tannin and total phenolic content from mashing to evaporation, but for ABTS method, pasteurization showed significantly the lowest antioxidant activity.

When the product, raw materials and waste products were evaluated, pasteurized nectar showed the lowest values for total phenolic content, total flavonoid content, total anthocyanin content, total tannin content and all antioxidant activity assays as 1005.31 mg GAE/100g DW, 1023.87 mg QE/100g DW, 26.3 mg cyn-3-gly/100g DW, 86.6 mg CE/100g DW and 4313.41 (CUPRAC), 1843.50 (DPPH), 1650.18 (FRAP) and 1177.36 (ABTS) mg TEAC/100g DW, respectively. There was no significant difference between press cake and precipitate for all analysis, except for total anthocyanins. Peel showed the highest value for total phenolic content, total flavonoid content, total tannin content and all total antioxidant activity methods, 18029.17 mg GAE/100g DW, 23005.89 mg QE/100g DW, 1563.4 mg CE/100g DW and 90876.26 for CUPRAC, 42884.98 for DPPH, 26622.14 for FRAP and 51100.85 mg TEAC/100g DW for ABTS, respectively. Whereas for total anthocyanin content, pomegranate arils showed the highest value as 176.6 mg cyn-3-gly/100g DW.

According to polymeric color (%) analysis, pasteurization showed higher value than peel and raw material but there was no significant difference.

As a result of phenolic profiling by HPLC analysis, gallic acid, ferulic acid, quercetin 3-β-D-glucoside, delphinidin 3,5-di-O-glucoside and cyanidin 3-O-glucoside were found for all samples including processing steps, end products and waste products.

According to total phenolic content analysis of bioavailability samples, peel showed highest postgastric (PG), solution entering the dialysis tubing which is serum fraction (IN) and solution not entering the dialysis tubing which is colon fraction (OUT) values, however, IN % residue values for total antioxidant activity were the lowest. IN % residue value of pasteurized nectar for DPPH were higher than other samples. Extract values were much higher than IN values of all samples.

In conclusion, conditions of processing steps such as mashing and pasteurization can be optimized to protect health effects of pomegranate by means of phenolic content, flavonoid content, tannin content and antioxidant activity. Waste products, especially pomegranate peel, can be used as functional ingredients of food formulations or dietary supplements. By this way, the wastes can gain economic value besides providing health effects of pomegranate for longer durations to consumers in a wide range of products.

NARIN NEKTARA İŞLENMESİNDE POLİFENOLLERDE VE ANTİOKSİDAN AKTİVİTEDEKİ DEĞİŞİMLER

ÖZET

Nar (*Punica granatum*), zengin antioksidan potansiyeli ve fenolik içeriği nedeniyle sağlık açısından önemli bir meyvedir. Narın içerdiği önemli fenolik bileşenler, antosiyaninler, flavonol glikozitleri, prosiyanidinler, ellajik asit ve türevleri gibi fenolik asitlerdir. Yüksek antioksidan kapasitesi tanenler, flavonoidler ve antosiyaninler gibi değerli fenolik bileşenleri içermesi nedeniyledir. Fenolik bileşenler ve polifenoller antioksidan aktiviteleri nedeniyle önemli antimutajenik ve antikanserojenik özelliklere sahip olup kalp ve damar hastalıklarına karşı da koruyucu etkilere sahiptir.

Narın bu önemli bileşenleri ve bileşenlerinin yüksek antioksidan aktivitesi nar, nar ürünleri ve nardan elde edilen meyve sularına olan ilgiyi son yıllarda arttırmıştır. Literatürde, narın yüksek antioksidan aktiviteye sahip olduğunu gösteren birçok çalışma bulunmaktadır; ancak bilindiği üzere gıdaların fenolik içeriği ve antioksidan aktivitesi yetiştirme, işleme ve depolama koşulları gibi birçok faktöre bağlıdır. Nar suyu üretimi boyunca da fenolik bileşenlerde değişimler veya kayıpların olabileceği belirtilmektedir. Nar suyu üretiminin proses basamaklarında fenolik içerik ve antioksidan aktivitenin değişimiyle ilgili bazı çalışmalar bulunmasına rağmen, nar taneleri, nar kabuğu ve ham maddeden ürüne kadar bütün proses basamaklarını içeren kapsamlı bir araştırma bulunmamaktadır. Bu çalışmanın temel amacı; pastörize nar nektarı üretimi boyunca proses basamaklarının (mayşeleme, presleme, soğutma, pastörizasyon, enzim uygulaması, durultma, ultrafiltrasyon, evaporasyon, nektar, nektar pastörizasyonu) ve ayrıca ham madde, nar taneleri, nar kabuğu, presleme sonrası posa, durultma sonrası tortu gibi atık ürünlerin ve elde edilen son ürünlerin toplam fenolik içeriği, toplam flavonoid içeriği, toplam tanen içeriği, toplam antioksidan aktivitesi, (%) polimerik renk, önemli fenolik bileşenler ve antosiyaninlerdeki değişim ve kayıpların araştırılması ve karşılaştırma yapılmasıdır. İkincil olarak ise; insan metabolizmasına olası etkileri daha net görebilmek için, hammadde, yan ürünler ve son ürünlerde potansiyel biyoyararlılıkların karşılaştırılmasını *in vitro* sindirim sonrası fenolik madde içeriği, antosiyanin içeriği ve antioksidan aktiviteyi esas alarak yapmaktır.

Örnekler, endüstriyel ölçekte nar nektarı üretimi yapan Karaman'daki meyve suyu işleme tesisinden temin edilmiştir. Örnekler iki farklı üretimden iki paralel olacak şekilde toplanmıştır. Laboratuvarda -80^{o}C'de depolanmış ve sıvı azot kullanılarak öğütülmüş ve analizler için hazır hale getirilmiştir. Nem analizi vakumlu etüv metoduyla 70^{o}C ve 600 kPa basınç altında 6 saat boyunca bekletilerek yapılmıştır. Ekstraksiyon, %0.1 formik asit içeren %75 metanol:su çözeltisi kullanılarak gerçekleştirilmiştir. Her analiz için standart kalibrasyon grafikleri hazırlanmıştır.

Toplam fenolik içeriği, toplam tanen içeriği, toplam antosiyanin içeriği sırasıyla Folin-Ciocalteu metodu, vanillin metodu ve pH diferansiyel metodu kullanılarak

ölçülmüştür. Toplam antioksidan aktivite, 2,2-difenil-1-pikrilhidrazil radikal yakalama kapasitesi (DPPH), Kuprik İyon İndirgeme Kapasitesi (CUPRAC), Plazmanın Demir İndirgeme Antioksidan Gücü (FRAP) ve 2,2'-azinobis-3 etilbenzotiyazolin-6-sulfonik asit radikal giderme aktivitesi (ABTS) metotları kullanılarak analizlenmiştir. (%) Polimerik renk ölçümü potasyum metabisülfit metodu kullanılarak yapılmıştır. Önemli fenolik bileşenler (gallik asit, kateşin, kuersetin-3-β-D-glukozit, kuersetin-3-galaktozit, ferulik asit, neoklorojenik asit ve p-koumarik asit) ve önemli antosiyaninler (siyanidin 3-O-glukozit, siyanidin 3,5-di-O-glukozit, delfinidin 3-O-glukozit, delfinidin 3,5-di-O-glukozit, pelargonidin 3-O-glukozit ve pelargonidin 3,5-di-O-glukozit) Ters Faz Yüksek Performanslı Sıvı Kromatografisi/Fotodiyot Dizisi Detektör (RP-HPLC/PDA) kullanılarak araştırılmıştır.

Nar bütün mevsimlerde yetişmemektedir; fakat yeni ürünlere işlendiğinde daha uzun süre tüketilebilmektedir. Bu nedenle, narın biyoyararlılığının incelenmesi gerekmektedir. Bu çalışmada sadece ürün, nar taneleri, ham madde için değil, ayrıca kabuk, presleme sonrası posa ve durultma sonrası tortu gibi yan ürünler için de *in vitro* biyoyararlılık araştırılmıştır. Enzimler ve *in vitro* sindirim metotu kullanılarak insan vücudundaki sindirim sistemi taklit edilmiş ve prosesin biyoyararlılık üzerine etkisi incelenmiştir. Biyoyararlılık örnekleri için, DPPH metodu kullanılarak toplam antioksidan aktivitesi, Folin-Ciocalteu yöntemi ile toplam fenolik içeriği analizi, pH diferansiyel metodu ile toplam antosiyanin içeriği analizi ve RP-HPLC/PDA kullanılarak fenolik bileşen ve antosiyanin analizi yapılmıştır.

Analiz sonuçlarının tümü istatistiksel olarak Sosyal Bilimler için İstatistik Paketi (SPSS) 16.0 versiyonu yazışımı yardımı ile tek yönlü varyans analizi (ANOVA), uygulanması sonrasında Duncan Yeni Çoklu Aralık Testi ile 0.05 önem derecesinde değerlendirilmiştir. Sonuçlar, mg eş değerleri/100 g kuru madde (KM) olarak belirtilmiştir. Her bir analiz her örnek için üç kez tekrarlanmış ve sonuçlar ortalama değer ± standart sapma olarak verilmiştir.

Genel olarak, nar kabuğu, diğer örneklerden daha yüksek fenolik içerik ve antioksidan aktivite göstermiştir. Nar kabuğunun atılmasında ortaya çıkan kayıplar nedeniyle ham maddeden mayşelemeye geçişte bir azalma olmuştur. Pastörize edilmiş nektar, en düşük değerleri göstermiştir ve nektarın pastörizasyonu sonuçları önemli derecede değiştirmemiştir.

Ham maddeden ürüne üretim boyuncaki değişime göre, ham madde toplam fenolik içeriği, toplam flavonoid içeriği, toplam tanen içeriği ve tüm antioksidan aktivite analizleri için sırasıyla, 11161.5 mg gallik asit eş değeri (GAE)/100g KM, 14127.1 mg kuersetin eşdeğeri (QE)/100g KM, 1883.1 mg kateşin eşdeğeri (CE)/100g KM ve 60677.1 (CUPRAC), 26327.0 (DPPH), 15754.9 (FRAP) and 30762.6 mg Troloks eş değeri antioksidan kapasitesi (TEAC)/100g KM (ABTS) olarak en yüksek değeri göstermiştir. Soğutma basamağı toplam antosiyanin içeriği değerini 162.0 mg cyn-3-gly/100g KM'ye arttırmıştır. Toplam antosiyanin içeriği dışında tüm analizlerde ham maddeden mayşelemeye azalma görülmüştür. Ürün, toplam flavonoid içeriği hariç en düşük değerlere sahiptir. CUPRAC, toplam tannin ve toplam fenolik içeriği için mayşelemeden evaporasyona kadar değişim görülmemiştir; fakat pastörizasyon ABTS için önemli derecede en düşük antioksidan aktiviteyi göstermiştir.

Ürün, ham maddeler ve yan ürünler değerlendirildiğinde ise, pastörize nektar toplam fenolik içeriği, toplam flavonoid içeriği, toplam antosiyanin içeriği, toplam tanen içeriği ve tüm antioksidan aktiviteleri için, sırasıyla, 1005.31 mg GAE/100g KM,

1023.87 mg QE/100g KM, 26.3 mg cyn-3-gly/100g KM, 86.6 mg CE/100g KM ve 4313.41 (CUPRAC), 1843.50 (DPPH), 1650.18 (FRAP) ve 1177.36 (ABTS) mg TEAC/100g KM ile en düşük değerleri göstermiştir. Presleme sonrası posa ve durultma sonrası tortu arasında toplam antosiyanin hariç bütün analizlerde önemli bir fark görülmemiştir. Kabuk, toplam fenolik içeriği, toplam flavonoid içeriği, toplam tanen içeriği ve tüm antioksidan aktivite metotları için sırasıyla 18029.17 mg GAE/100g KM, 23005.89 mg QE/100g KM, 1563.4 mg CE/100g KM ve 90876.26 (CUPRAC), 42884.98 (DPPH), 26622.14 (FRAP) ve 51100.85 mg TEAC/100g KM (ABTS) ile en yüksek değerleri göstermiştir. Toplam antosiyanin içeriği içinse nar taneleri 176.6 mg cyn-3-gly/100g KM ile en yüksek değere sahiptir.

(%) Polimerik renk analizine gore, pastörizasyon nar kabuğu ve ham maddeden daha yüksek değer göstermiştir; fakat aralarında önemli fark görülmemiştir.

HPLC ile fenolik bileşen analizi sonucunda, gallik asit, ferulik asit, kuersetin 3-β-D-glukozit, delfinidin 3,5-di-O-glukozit ve siyanidin 3-O-glukozit, proses basamakları, yan ürünler ve son ürünleri içeren bütün örnekler için bulunmuştur. En yüksek siyanidin 3-O-glukozit ve pelargonidin 3,5-di-O-glukozit konsantrasyonu pastörizasyon basamağında görülürken; en yüksek delfinidin 3,5-di-O-glukozit konsantrasyonu soğutma basamağındadır. Nar kabuğu gallik asit, kuersetin-3-galaktozit ve kuersetin-3-β-D-glukozitin en yüksek değerlerine sahip olmuş; fakat en yüksek ferulik asit değeri ham maddede görülmüştür.

Biyoyararlılık örneklerinin toplam fenolik içerik analizinin değerlendirilmesinde kabuğun, mide sonrası (PG), diyaliz tübünde giren çözelti (IN) ve diyaliz tübüne girmeyen çözelti (OUT) değerleri en yüksek iken toplam antioksidan aktivitesinin kalan % IN değerleri en düşüktür. Bütün örnekler için IN değerleri, ekstrakt değerleriyle karşılaştırıldığında çok düşüktür. Potansiyel antosiyanin biyoyararlılığı potansiyel fenolik biyoyararlılığından çok daha düşük bulunmuştur. Antosiyaninler gastrik koşullara az da olsa dayanıklı olmasına rağmen, çok düşük seviyelerde seruma (IN fraksiyonuna) geçebilmiştir. Fenoliklerin biyoyararlılığı proses boyunca değerlendirildiğinde, sindirim boyunca kabuğun yüksek fenolik değerleri önemli derecede korunmuştur. Konsantre edilmiş meyve suyu, durutlma sonrası tortu ve presleme sonrası posa gibi ürünler fenoliklerin bazı korunmuş değerlerine sahiptir; fakat son ürün olarak pastörize nektar, sindirim boyunca önemli miktarda fenoliklerini kaybetmiştir. Isıl uygulamalar, evaporasyon ve pastörizasyonun *in vitro* sindirimden sonra antioksidan aktiviteyi olumsuz etkilediği bulunmuştur.

Antosiyaninlerin biyoyararlılığı değerlendirildiğinde, nar taneleri en yüksek değere sahip olmuş; fakat sindirim boyunca korunmuş en yüksek değerler özellikle gastrik koşullardan sonra ve serum için konsantre ve pastörize nektarda elde edilmiştir. Artık ürünlerin antosiyanin biyoyararlılığı gastrik koşullardan sonra %50 ve serum fraksiyonunda %2-3 olmuştur.

Sonuç olarak, proses boyunca üretim basamaklarında bileşenlerin, antioksidan aktivitenin ve biyoyararlılığın değişimi gözlenmiştir. Narın nektara işlenmesi boyunca ham madde ve nar taneleri yanında nar kabuğu başta olmak üzere proses boyunca posa ve tortu gibi atılan ürünlerin önemli polifenolik bileşen ve antioksidan aktiviteye sahip olduğu görülmüştür.

Ham maddedin mayşelenmesi ve pastörizasyon gibi proses basamakları narın fenolik bileşen içeriği, flavonoid içeriği, tanen içeriği ve antioksidan aktivitenin korunması ve sağlık etkilerinden faydanılması için geliştirilebilir.

Yan ürünler, özellikle nar kabuğu, diyet takviyelerinde veya gıda formülasyonlarında fonksiyonel bileşen olarak kullanılabilir. Böylece, yan ürünlerin hem ekonomik değer kazanmaları hem de sağlığa etkilerinin uzun süreli olarak sağlanması gerçekleştirilebilir.

1. INTRODUCTION

Pomegranate (*Punica granatum*) is one of the important fruits due to its high antioxidant activity. It is cultivated in Afghanistan, China, India, Iran, Japan, Mediterranean countries, Russia and USA (Alighourchi et al., 2008). It is usually consumed as fresh fruit, beverage and food products such as wine and sour sauce and used in herbal medicines and dietary supplement as ingredient. Phytochemicals can be obtained from different parts of fruit such as peel, juice and seeds (Elfalleh et al., 2011).

In the last years, the interest to determine dietary sources of antioxidant phenolics have increased and red juices have gained attention because of their antioxidant activity. Although pomegranate has a traditional importance for years as a medicinal plant; due to obtaining data from research which show its anticarcinogenic, antioxidant, antimicrobial, antiviral and antitumoral properties, pomegranate juice has become more popular in the recent years (Gil, et al., 2000; Tzulker et al., 2007).

Pomegranate juice has significant compounds, some of which are antioxidants, such as anthocyanins, ellagic acid, phytoestrogenic flavonoids, tannins and organic acids (Mirsaeedghazi et al., 2011). Pomegranate peel is also a powerful source of phenolic compounds such as tannins, catechin, quercetin, anthocyanin and ferulic acid, which have biolological activities such as reducing oxidation, microbial growth, risk of some cancers and cardiovascular diseases by its rich polyphenols (Opara et al., 2009).

Pomegranate is a seasonal fruit, so the suitable conditions should be selected to preserve its content and antioxidant activity (Mirsaeedghazi et al., 2011). Therefore, bioavailibility should be determined to research new suggestions to be consumed in a longer time. Pomegranate can be processed to a wide variety of products, one of which is pomegranate nectar. Pomegranate juice production is thought to cause some changes or losses in polyphenol content and antioxidant acitivty of pomegranate, however, there has not been any research about the effect of all production steps.

The aim of this study was to investigate the effect of all processing steps of pasteurized pomegranate nectar production on polyphenols and antioxidant activity besides researching changes and losses in pomegranate peel, raw material and pomegranate arils. Moreover the other purpose of this study was to determine and understand *in vitro* potential bioavailability of pomegranate arils, raw material, the product and waste products.

This master thesis consists of literature review, materials and methods, results and discussion, and conclusions and recommendations sections. In the literature part, general properties of pomegranate, pomegranate production in the world, production and consumption in Turkey, health effects of pomegranate, pomegranate products, chemical composition of pomegranate, important phenolic compounds and phenolic compounds of pomegranate, bioavailability and potential bioavailability of pomegranate and studies related to this subject were reviewed. The materials and methods part included information about used materials, chemicals and methods. Evaluation of the results statistically and comparision with literature were performed in the results and discussion section. In the conclusion, the general results and recommendations were reported.

2. LITERATURE REVIEW

2.1 Pomegranate

There are two species of Punica: *Punica protopunica* and *Punica granatum*. *P.granatum* is grown in tropical and subtropical regions and *P.protopunica* is grown in Socotra Island. Several types of pomegranate have been produced with differences in their shapes, color, thickness, peel and aroma. Pomegranate can be produced on different soils and the trees which are in deep, abundant and alluvial soils give higher yield. Pomegranate can be grown in a climate with cool winters and hot summers because during ripening of pomegranate; it needs a hot and dry climate (Adsule and Patil, 1995).

Punica granatum L. (Punicaceae) has been an important plant in Asia, Mediterranean and Europe since ancient history. In Egyptian culture, pomegranate was a sign of abundance and desire. It was claimed in Ebers'papyrus (in 1500 B.C.) that pomegranate was used as a treatment for tapeworm or parasitic contaminations. It was expressed in Greek mythology as "fruit of the dead" (Jayaprakasha et al., 2006).

According to their structures, pomegranates are divided into varieties such as Devedişi, Çekirdeksiz, Zivzik Çekirdeksiz, Kadı, Lefon, Keban, Hicaz and Misk. They are separated to classes based on their properties such as Class I and Class II. They are separated to different heights such as small, medium, large and too large by their weight or the largest equatorial diameter (TS 4953, 1986).

Pomegranate has four main parts, basically: Peel, seeds, arils and membrane (between peel and arils). Pomegranate contains about 60-67% seeds and 33-40% peel. Juice can be made from 76-85% of arils and 45-61% of whole fruit (Adsule and Patil, 1995). Arils includes 85% water, 10% total sugars, principally fructose and glucose, 1.5% pectin and organic acids like ascorbic acid, citric acid and malic acid (Martos et al., 2011).

2.2 Pomegranate Production and Consumption

Pomegranate can be grown in several countries which have tropical or subtropical regions. In Turkey, it is usually consumed and produced. Besides consuming as a fruit, it can be used for production of different products or adding in fruit juices.

2.2.1 Pomegranate production in the world

Pomegranate can be grown in many countries including in Middle East, the Mediterranean region, and other areas in Asia. These countries are China, Afghanistan, Pakistan, Bangladesh, Iran, Iraq, the east India, Russia, Japan, Malaysia, the United States, the drier parts of Southeast Asia and Saudi Arabia. There are some fruit gardens in Israel on the coast and in the Jordan Valley (Shi and Moy, 2005; Martos et al., 2010).

Turkey, China, India, Iran, Afghanistan, Spain, Egypt, Israel and Tunis are the countries which export pomegranate. The important pomegranate varieties grown commercially in these countries are Wonderful, Mollar, Tendral, Schahvar, Robab, Hicaznar, Zehri, Gabsi, Alandi and Ganesh (Yazıcı and Sahin, 2007).

In spite of its common production, no published information about the worldwide production and and the statistics for each country is available. There are some studies in which researches have given the information related to production or consumption about their country. The amounts of pomegranate production according to those information gathered for different years are shown in Table 2.1.

2.2.2 Pomegranate production and consumption in Turkey

Pomegranate is commonly produced and consumed in Turkey. Not only as a fruit or in fruit juice production, it is also used in manufacturating of different products.

Pomegranate production and consumption have been increasing in Turkey. After 2007, the production was higher than 100000 tons and the consumption per person was higher than 1 kg.

Amounts of pomegranate production according to regions in decreasing order are: Mediterranean (61.8%), Aegean (23.3%) and Southeastern Anatolia (9.1%). The export values for pomegranate have increased especially in last years.

Table 2.1: Pomegranate production in the world (Yazıcı and Sahin, 2007).

Country	Year	Production (Tonnes)
India	2005	792 500
Iran	2005	705 165
China	2004	180 000
Turkey	2006	90 737
Syria	1996	62 000
Tunis	1999	50 000
USA	2004	54 000
Pakistan	2005	49 900
Morocco	2003	45 900
Egypt	1993	33 700
Spain	2006	40 000
Azerbaijan	2000	40 000
Afghanistan	2003	28 000
Tajikistan	2000	20 000
Israel	2006	15 000
Jordan	2000	4 419
Sri Lanka	2000	1906
Portugal	1993	1 810
Yemen	1995	7 110
Greece	1993	6 000
Mexico	2001	3 529
The Greek Cypriot State	1997	700
Palestine	2002	411
Italy	1995	200

Pomegranate is exported to countries such as Germany, Russia, Netherlands and Ukraine. Hicaznar is the most commonly exported pomegranate variety from Turkey to Europe. It is popular due to its red peel, dark reed arils and sourish taste, besides its suitability for preservation. The other varieties grown in Turkey are: Çekirdeksiz, Silifke Aşısı, Katırbaşı and Lefan (Yazıcı and Sahin, 2007). The amount and commercial value of export for pomegranate in 1998-2007 is shown in Table 2.2.

Table 2.2: The amount and commercial value of export in 1998-2007 (Yazıcı and Sahin, 2007).

Year	Export (tonnes)	Commercial Value (USD)
1998	2.913	2.183.709
1999	4.321	2.499.461
2000	3.591	2.012.617
2001	7.869	3.371.543
2002	7.336	4.238.930
2003	9.507	6.662.181
2004	11.495	7.335.486
2005	11.447	9.435.868
2006	10.916.653	11.209.071
2007	13.731.574	16.860.976

Pomegranate production and consumption in Turkey in 2000-2010 are shown in Table 2.3.

Table 2.3: Pomegranate production and consumption in Turkey (TURKSTAT, 2010).

Year	Number of Trees		Production (Tonnes)	Consumption per Person (kg)
	Fruit Giving	No Fruit Giving		
2000	2 485	809	59 000	0.75
2001	2 530	840	60 000	0.70
2002	2 670	855	60 000	0.69
2003	3 190	1 100	80 000	0.91
2004	3 200	1 220	73 000	0.78
2005	3 220	1 409	80 000	-
2006	3 136	1 502	90 737	-
2007	3 611	3 367	106 560	1.19
2008	4 017	5 929	127 760	1.24
2009	5 092	5 794	170 963	1.49
2010	6 431	5 679	208 502	-

Most of the pomegranate in Turkey is grown in Mediterranean and Aegean cities such as Antalya, Mersin and Aydın. The most pomegranate growing cities in Turkey are shown in Table 2.4, about 38% of the pomegranate in Turkey is grown in Antalya.

Table 2.4: The most important cities in Turkey that produces pomegranate (Yazıcı and Sahin, 2007).

City	Area (hectare)	Production (tonnes)	Production (%)
Antalya	1.882	28.053	38.4
Mersin	309	8.334	11.4
Aydın	542	6.469	8.9
Denizli	415	5.979	8.2
Hatay	138	4.385	6.0
Siirt	900	3.159	4.3
Adana	118	2.272	3.1
İzmir	75	2.058	2.8
Muğla	131	1.512	2.1

Pomegranate is commonly used as a fruit juice or an ingredient in mixed fruit juices in Turkey. Its utilization has been increasing since 2005. The amounts of fruits used for fruit juice production between 2001-2008 are shown in Table 2.5.

Table 2.5: The amount of fruits used for fruit juice manufacturing in Turkey (MEYED, 2008).

Fruit	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**
Cherry	28.2	9.9	54.7	35.7	37.1	52.2	72.6	54.6
Apricot	37.2	13.9	34.8	24.8	30.8	36.1	38.2	74.9
Peach	31.5	26.2	51.5	30.2	75.9	65.3	90.1	118.8
Apple	272.9	244.5	341.5	338.0	409.2	282.9	356.8	333.8
Orange	12.6	31.7	28.3	46.2	33.1	37.8	53.3	63.9
Pomegranate					17.6	46.6	57.5	49.5
Carrot							30.6	30.7
Grape					10.9	8.4	18.3	16.9
Strawberry							4.1	7.7
Grapefruit								5.5
Quince							7.5	4.5
Tomato					4.6	4.9	3.9	4.4
Lemon								2.7
Others	6.0	19.3	10.5	16.9	10.2	47.9	4.3	3.2
Total	388.4	345.5	521.3	510.3	629.4	582.1	737.2	771.1

2.3 Pomegranate in Health and Disease

Pomegranate has valuable bioactive compounds which give this fruit several functional and medicinal properties. Besides being a powerful antioxidant fruit, it can act as antitumoral, antidiabetic, antihepatoxic and antimicrobial. It can protect cardiovascular health and improve oral and skin health. It prevents from Alzheimer's disease and develops sperm quality. The inhibitory effects of pomegranate extracts are due to their phenolic, anthocyanin and tannin content (Martos et al., 2010). The researches about the effect of pomegranate on human health have not been completed yet.

Some *in vivo* studies in human and animal have investigated the effect of pomegranate on protection from LDL oxidation and atherosclerosis. The studies reported that pomegranate showed some effects such as reducing total cholesterol, LDL cholesterol, fatty acids, triglycerides, lipid oxidation levels and increasing plasma antioxidant capacity. Punicic acid, which is found in pomegranate seed oil shows an *in vivo* anti-inflammatory effect by inhibiting lipid peroxidation. There are several studies focusing on the antitumoral or anticancer properties of pomegranate. Anthocyanins and punicalagin are the most effective compounds. Anthocyanins decrease colon cancer, however rutin, epicatechin and chlorogenic acid do not have a powerful effect. In some of the findings, fermented pomegranate products such as

wine and seed oil inhibited oxidation and breast cancer cell generation. Pomegranate extract ensured an inhibition on growth of prostate cancer cells according to dose (Martos et al., 2010).

It prevents from cancer by targeting many proteins in the cell-communication pathway. Molecular targets of pomegranate are coronary heart disease, skin cancer, brain disorders, inflammation, aging, AIDS, prostate cancer and colon cancer. Fermented pomegranate juice showed anticancer properties on human breast cancer cells and whole pomegranate seed was more chemopreventive than polyphenols (Shishodia et al., 2006). Pomegranate juice has been suggested in the treatment of acquired immune deficiency syndrome (AIDS) due to its bioflavonoids and inhibition of lipoxygenase. Pomegranate is replaced in nine herbs used for treatment of AIDS in Japan (Perez-Vicente et al., 2002).

Pomegranate has also antidiabetic properties because of its polyphenol content. Glucose uptake is increased by polyphenols and glycemia is decreased. Gluconeogenesis is decreased, glucose uptake and insulin release are activated (Martos et al., 2010).

There are some studies about the effect of pomegranate on improving skin health. They generally investigated its effect on human skin against UVA and UVB damage. Pomegranate products could be effective on UV-irradiated pigmentation on brown skin and whitened the skin after oral administration. They inhibited the generation of melanocytes and melanin production. Pomegranate extract can prevent from UVA damage (Martos et al., 2010).

Polyphenolic flavonoids in pomegranate help to prevent from gingivitis and improve oral health. Washing with pomegranate extract decreased activities of α-glucoside and increased activities of ceruloplasmin which is an antioxidant enzyme in saliva. Tannins decrease α-amylase activity and this ensures an acidogenic food source for carcinogenic microorganisms in the mouth (Martos et al., 2010).

Some studies have showed the antimicrobial effect of pomegranate extracts. Methanolic extracts could inhibit the growth of *Staphlylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhi*. Chloroform, ethanol and water extract of pomegranate were very effective on *E.coli* O157:H7. There were also researches about antimicrobial activity of pomegranate peel on

microorganisms. 80% methanolic extract of pomegranate peel was effective on *Listeria monocytogenes*, *S.aureus*, *E.coli* and *Yersinia enterocolitica* (Martos et al., 2010). Punicalagin which was present in pomegranate peel showed antimicrobial activity against *Candida albicans*. In a study investigating fungistatic activity of pomegranate peel, 69 test organisms were studied. It inhibited the growth of *Penicillium citrinum* for 8 days, *P. patulum* for 4 days, and *P. roquefortii* and *Aspergillus ochraceus* for 3 days, but it showed no effect on *A. flavus* and *A. parasiticus*. It showed some important or limited effects on viruses. Extracts obtained from fruit and stems were used in treatment for viral diseases such as influenza. Pomegranate compounds such as flavonoids, tannins, caffeic acid derivatives, terpenoids, and saponins showed antiviral effects to Herpes Simplex Virus (HSV) and Respiratory Syncytial Virus (RSV) *in vitro* or *in vivo* (Jayaprakasha et al., 2006).

According to some researchers pomegranate juice has effect on sperm quality. It can increase sperm concentration, sperm motility, cell density and decrease abnormal sperm rate (Martos et al., 2010).

In some studies about the effects of pomegranate extract on obesity in animals showed that a diet including 20% of the extract for 37 d decreased feed consumption and weight (Martos et al., 2010).

It has also several other properties on health. It increases urine and it is against arthritis and hypertension. Pomegranate peel, flowers and juice is protecting from diarrhea and dysentery. Due to its positive effects on health, peel, flowers, seeds and fruit of pomegranate can be used as a medicine (Vardin and Abbasoglu, 2004). In India, Tunisia and Guatemala, dried pomegranate peels are boiled and used against many health problems such as astringents, diarrhea and ulcers.

Pomegranate derived products are used for cosmetic beautification, hormone replacement therapy, solution of allergic symptoms, cardiovascular protection, oral hygiene, ophthalmic ointment, weight loss as a soap, and as an adjunct therapy to increase bioavailability of radioactive dyes during diagnostic imaging (Lansky and Newmana, 2007). Pomegranate also prevents from neurological damage, ulcers, arterial plaques but these effects have not been justified definitely. Few studies have shown its effect, and more studies should be done to prove its effects, certainly.

Pomegranate extracts have been improved as a dietary supplement due to their bioactive compounds. There are also some studies searching if pomegranate extracts have toxic effect or not, but there is no evidence that they are toxic.

The inhibition or inactivation mechanisms depends on several factors such as genetic systems, enzymes, protein synthesis, cell membrane, cell wall, growth and climatic conditions, besides the part of the fruit which directly effects the antioxidant properties of pomegranates (Martos et al., 2010).

2.4 Pomegranate Products

Pomegranate fruit, especially arils, can be used for several purposes in the form of different products. They can give a red color for juices or different taste for sauces and help to obtain new and useful products.

2.4.1 Pomegranate juice and concentrate

Pomegranate juice can be obtained from whole fruit or arils. Using whole-fruit gives 42% yield, whereas using grains give a yield around 70%. Pomegranate concentrate has an important potential to be used in fruit-based beverages (Adsule and Patil, 1995). In Azerbaijan, Georgia and Central Asia, pomegranate juice is used with other juices to improve different tastes and obtain citric acid and vinegar (Vardin and Abbasoglu, 2004). Pomegranate juice or concentrate is preferred, especially, for mixed fruit juices. The statistics about consumption and production of pomegranate juice for the last years support this.

2.4.2 Sour pomegranate sauce

Sour pomegranate sauce is produced by pressing fruit, clarifying the juice and then darkening under vacuum, respectively. It is used to give taste to salads and foods. According to TSI (Turkish Standards Institution), it should not contain saccharose and any particles of fruit for its sensory properties (Vardin and Abbasoglu, 2004).

2.4.3 Canned pomegranate arils

It is a new application for pomegranate which became popular in the last years. Besides using directly as a canned product, it can be used in confectionery industry by increasing its sugar content, (Vardin and Abbasoglu, 2004).

2.4.4 Dried pomegranate arils (Anardana)

Arils can be packaged in plastic bags by modify atmosphere packaging. Pomegranate arils which are stored by deep freezing in suitable packages are exported to Middle East countries. “Anardana”, which is produced by drying pomegranate arils in India, is used to improve taste as an acidifier in foods (Vardin and Abbasoglu, 2004).

2.4.5 Pomegranate seed

It is known that pomegranate seeds contain about 20.8% oil. The residual oil after obtaining vegetable oil, is a very significant fodder additive which increases the yield of milk for animals. Due to their essential lipid content, these lipids are considered to be healthy. They prevent cardiovascular diseases and decrease total cholesterol and HDL (High Density Lipoprotein). They can be used in pharmacy and cosmetic industry and exported to some countries from Turkey (Vardin and Abbasoglu, 2004).

2.4.6 Pomegranate wine

Pomegranate wine is produced from whole fruit without breaking. Sugar is used to obtain 22-23^{o} Brix and potassium metabisulfite is used to protect from microorganisms. Wine yeast is added for fermentation and the wine is matured. It is pasteurized, bottled and the bottles are cooled (Adsule and Patil, 1995).

2.4.7 Pomegranate syrup

Pomegranate syrup is produced by pasteurization or by using sodium benzoate. The syrup has a purplish-red color and delicious taste with 60^{o} Brix and acidity by addition of 1.5% citric acid (Adsule and Patil, 1995).

2.4.8 Other products

There is a special drink in France, expressed as “grenadine”, which can be made from pomegranate juice.

Pomegranate juice is boiled with soft wheat and dried as small buckthorns in some villages in Turkey. It is named as “buckthorn” and can be stored for a long time (Vardin and Abbasoglu, 2004).

Pomegranate jam is produced by concentrating juice, using sugar and heating for a long period (Adsule and Patil, 1995).

In Saudi Arabia, the juice sacs can be frozen or the extracted juice can be concentrated to use in the future (Shi and Moy, 2005).

2.5 Chemical Composition of Pomegranate

Pomegranate is a nutritious fruit source due to its important chemical composition and mineral content. Pomegranate have carbohydrates and minerals (Ca, Fe, S., etc.) as important nutrients. The general chemical compositions and the detailed vitamin and mineral contents of pomegranate according to different studies are shown in Table 2.6 and Table 2.7, respectively.

Table 2.6: Chemical composition of pomegranate (Adsule and Patil, 1995; Dallas 2003).

Component	Content		
	Jagtap et al.	Sood et al.	Dallas
Moisture (%)	78	77.0-78.2	80.97
Protein (%)	1.6	1.78-1.96	0.95
Fat (%)	0.1	1.72-2.11	0.30
Carbohydrates (%)	14.6-	17.5-20.0	17.17
Ash (%)	0.7	0.66-0.76	0.61
Crude fiber (%)	5.1	-	0.6
Pectin (%)	0.27	0.47-0.55	-
Total sugars (%)	-	6.2-9.0	-
Reducing sugars (%)	-	5.6-7.5	-
Nonreducing sugars (%)	-	0.1-3.3	-
Energy value (kcal/100g)	65.0	-	68

Table 2.7: Vitamin and mineral contents of pomegranate (Adsule and Patil, 1995; Dallas 2003).

Component	Content		
	Jagtap et al.	Sood et al.	Dallas
Thiamine	0.06	-	0.03
Riboflavin	0.1	-	0.03
Niacin	0.3	-	0.3
Vitamin C	16.0	5.3-7.7	6.1
Calcium	10.0	24-145	
Phosphorus	70.0	33-44	8
Iron	0.30	0.62-0.69	
Magnesium	12.0	-	3
Copper	0.17	-	
Sodium	4.0	-	
Potassium	17.1	-	259
Sulfur	-	25-28	

2.6 Important Phenolic Compounds

Pomegranate contain polyphenols; essentially flavonoids (flavonols, flavanols, anthocyanins), condensed tannins (proanthocyanidins), hydrolysable tannins (ellagitannins and gallotannins) and other phytochemicals.

2.6.1 Phenolic acids

Phenolic acids are significant compounds for fruit and vegetables. They have an important role in color, flavor and antioxidant capacity. For example, gallic acid derivatives and hydroxycinnamates are free radical acceptors and have important antioxidant properties. The stability of olive oil is dependent on phenolic antioxidants (Rice-Evans and Packer, 2003).

Phenolic acids can be divided into two groups: hydroxybenzoic acids (HBA) which derives from benzoic acid and hydroxycinnamic acids (HCA) which derives from cinnamic acid. They act as acids due to one carboxylic group in their molecule. They present in many fruit and vegetables and their distribution depends on species, cultivar and physiological stage (Rice-Evans and Packer, 2003).

The phenolic acid content can be affected by many factors such as the genetic background, the stage of the plant and the environmental and culture conditions. The changes are dependent on the arrangement of phenolic metabolism, enzyme activities and the gene expression.

Gene expression and enzyme activity are also dependent on external factors such as temperature, light. The enzymatic oxidation of phenolic compounds is important due to formation of the loss of compounds and unsuitable color and taste. The changes during food processing occurs due to oxidative degradation including PPO activities during such as enzymatic treatment, crushing, pressing, the release of free acids from conjugate forms and the formation of complex structures (Rice-Evans and Packer, 2003).

Their extraction is carried out by using ethanol or methanol-water solutions (80/20, v/v) at low temperatures. Chemical or enzymatic hydrolysis is needed for phenolic acids that are connected to cell wall to give insoluble forms. Under these conditions, supercritical carbon dioxide can be used for phenolic lipids and acidic methanol for

fruit anthocyanins. Paper chromatography, high-performance liquid chromatography (HPLC), thin-layer chromatography and column chromatography are used to determine phenolic acids since 1960s. Moreover, modern techniques such as infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry can be used for identification. HPLC is admitted to be the most useful method to identify plant phenolic acids in their native forms (Rice-Evans and Packer, 2003).

2.6.1.1 Phenolic acids of pomegranate

The results of the study which was published in 2002 by Poyrazoglu et. al., about the determination of the organic acids in pomegranate juice are presented in Table 2.8. The major organic acids present in pomegranate juice are gallic acid, ellagic acid, caffeic acid, chlorogenic acid, ferulic acid and p-coumaric acid.

Table 2.8: Major phenolic acids in pomegranate (Poyrazoglu et al., 2002).

Phenolic Acid	Composition (g/l)
Gallic Acid	4.55
Catechin	3.72
Quercetin	2.50
Chlorogenic Acid	1.24
Caffeic Acid	0.78
p-comaric Acid	0.06
Ferulic Acid	0.01

2.6.2 Anthocyanins

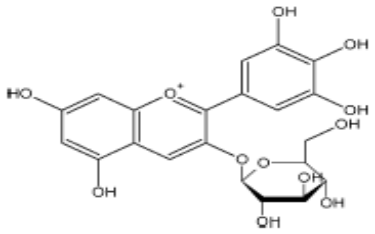
Anthocyanins give red, purple and blue colors to many fruits, vegetables and grains. There are 6 anthocyanidins and their structures can change according to glycosidic replacement at the 3rd and 5th positions (pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvinidin). There can be also some differences by acylation of the sugar groups with organic acids (Lee et al., 2005). There are many factors that can affect the content of anthocyanins such as cultivar, maturity, geographic location, light, temperature and various stresses (Fazzari et al., 2008).

2.6.2.1 Anthocyanins of pomegranate

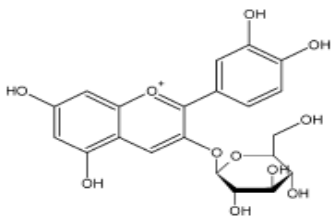
Principal anthocyanins found in pomegranate juice are: cyanidin 3-O-glucoside, cyanidin 3,5-di-O-glucoside, delphinidin 3-O-glucoside, delphinidin 3,5-di-O-glucoside, pelargonidin 3-O-glucoside, pelargonidin 3,5-di-O-glucoside which are shown in Figure 2.1. The number of hydroxylated groups, the nature and the number

of bonded sugars to their structure, the aliphatic or aromatic carboxylates bonded to the sugar in the molecule and the position of these bonds create the differences between them (Martos et al., 2010). Pelargonidin 3-O-glucoside and pelargonidin 3,5-di-O-glucoside are present mainly in the peel but smaller amounts in seed coats (Ozkal and Dinc, 1993). Delphinidin 3-O-glucoside and delphinidin 3,5-di-O-glucoside were not found in some of the studies, but cyanidin 3-O-glucoside and cyanidin 3,5-di-O-glucoside were present in considerable amounts (Gil et al., 2000; Mullen et al., 2007; Ozkal and Dinc, 1993; Turfan et al., 2008).

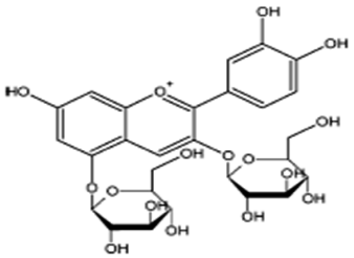
There can be a lot of factors that create differences in the phenolic and flavonoid content such as growing conditions (region, climate), variety, storage conditions and juice production methods (Martos et al., 2011).



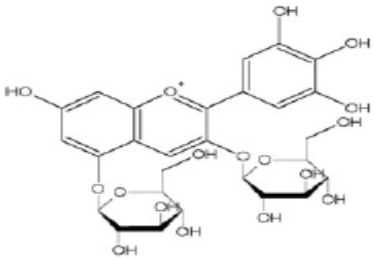
delphinidin 3-O-glucoside



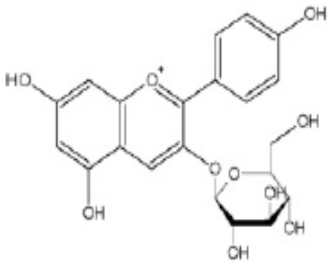
cyanidin 3-O-glucoside



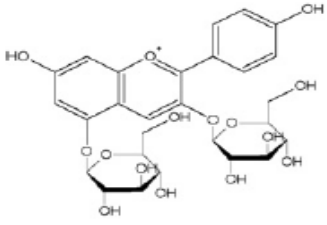
cyanidin 3,5-di-O-glucoside



delphinidin 3,5-di-O-glucoside



pelargonidin 3-O-glucoside



pelargonidin 3,5-di-O-glucoside

Figure 2.1: The major anthocyanins in pomegranate (Martos et al., 2010).

2.6.3 Tannins

Tannins are one of the important high molecular-weight polyphenol groups. They are separated to three groups: condensed tannins or proanthocyanidins (in tea, grapes, and cranberries), hydrolyzable tannins or ellagitannins (in raspberries and strawberries) and gallotannins. They can be used as anticeptics and astringents. The amount of tannins is important because they can precipitate protein, inhibit digestive enzymes and affect the utilization of vitamins and minerals.

2.6.3.1 Tannins of pomegranate

Hydrolyzable tannins which are the most important compounds of pomegranate consist of gallotannins, ellagitannins and gallayl esters such as punicalagin and punicalin. Pomegranate peel is a good source for hydrolysable tannins, especially, punicalin, pedunculagin and punicalagin. They differ from proanthocyanidins due to their chemical structure. Pomegranate also includes oligomeric ellagitannins with two to five glucose core molecules cross linked (Madrigal-Carballo et al., 2009).

2.6.4 Other compounds

Other phenolic compounds in pomegranate are organic acids (citric acid, malic acid, and oxalic acid), sterols, fatty acids, triglycerides and alkaloids (Elfalleh et al., 2011; Ozkal and Dinc, 1993). Pomegranate seed, especially, is a good source of fatty acids. It contains significant fatty acids such as linoleic, oleic, palmitic, stearic, linolenic, arachidonic and palmitoleic acids (Vardin and Abbasoglu, 2004). Pomegranate juice also consists of about all amino acids, especially valin and methionine in high values. In edible part 14.31% is carotenoid and 4.68% is carotene (Ozkal and Dinc, 1993).

2.7 Bioavailability

According to the expression of American Food and Drug Administration (FDA), bioavailability is absorption rate of the therapeutic substances or active compounds present in a drug and degree of getting used in the region they can be active. This description also includes active compounds in foods. By other meaning, bioavailability is the part of the food that can be available for use in normal physiological functions. There can be several factors that affect the potential bioavailability of antioxidants in humans which are shown in Table 2.9.

Bioavailability and bioaccessibility are different terms and they should not be used in the same meaning. Bioaccessibility is related to "The quantity of antioxidants which are released from the food matrix and presented to the intestinal brush border for transport into the cell". It is the proportion of the ingested antioxidants which are available to be used in the biological system. On the other hand, bioavailability is related to "The quantity of antioxidants which really pass during the cell membrane and can be used in the cell" (Wootton-Beard et al., 2011).

Table 2.9: Factors affecting bioavailability of antioxidants in humans (Porrini and Riso, 2008).

✓ Related to antioxidants
Chemical structure
Species/form
Molecular linkage
Concentration in foods
Amount introduced
Interaction with other compounds
✓ Related to the food preparation
Matrix characteristics
Technological processing
Presence of positive effectors of absorption: fat, protein, lecithin
Presence of negative effectors of absorption: fiber, chelating agents
Duration of storage
✓ Related to the host
Disorders and/or pathologies
Enzyme activity
Gender and age
Genetics
Hormonal status
Intestinal transit time
Microflora
Nutritional and antioxidant status
Physiological condition
Secretion of HCl
✓ External
Exposure to different environments
Food availability

Flavonoids are present in the form of aglycone and glucoside. Flavonoid glucosides are separated from sugar part before entering the intestine, whereas aglycones can freely pass through cell membranes. The absorbed flavonoids are transferred to liver and changed into distinct conjugation forms such as glucuronides, sulfates and methylated derivatives by being exposed to very various metabolic reactions. In

some studies, it was claimed that these conjugates are responsible for positive effects of flavonoids on health. On the other hand, absorption of flavonoids taken by nutrition was considered to be very low due to being connected to sugar structurally and not secreting any enzyme in the intestine wall to disintegrate glycosidic linkage. Therefore, only aglycones were expected to pass through the intestine wall by blood circulation, freely. Nevertheless, the last researches reported that some flavonoids showed bioavailability more than believed (Guven et al., 2010). For example, some studies have reported bioavailabilities of flavonoids connected to sugar in onion, apple, wine, grape, tomato puree, blackcurrant juice, orange juice, green and black teas and soy (Guven et al., 2010).

Anthocyanins are not stable under *in vitro* conditions. A decrease in IN value occurs due to differences in pH, oxygen and heating factors (McDougall et al., 2005a). Before 1995, it was believed that anthocyanins and some polyphenols could not be absorbed undamaged after oral ingestion, however, they were hydrolyzed to their aglycones by bacterial enzymes in lower gastrointestinal region. Then, it was claimed that the aglycones might be incompletely absorbed or might biotransformed by bacteria. There are not many studies about existence of anthocyanin aglycones in the blood circulation or urine of humans. Absorption of anthocyanins undamaged as glycosides has been described in several animal and human studies. According to these studies, anthocyanins stay undamaged in the circulation as glycoside forms (Fazzari et al., 2008).

Codigestion with foods does not considerably decrease the levels of serum (IN) bioavailable polyphenols. During digestion, polyphenols bind to food matrices and this protects unstable anthocyanins from degradation and they can diffuse into the IN sample (McDougall et al., 2005a).

The digestion conditions can be developed by combination *in vitro* absoption model (e.g. Caco-2 cells), which is a fast, safe and low cost for determination of the potential bioavailability of antioxidant compounds (Cilla et al., 2011).

2.7.1 Potential bioavailability of pomegranate juice

Pomegranate juice has a great health potential and this makes it important to evaluate biovailability of pomegranate polyphenols (Basu and Penugonda, 2009). There is limited research about bioavailability of pomegranate bioactive compounds,

absorption and metabolism of bioactive compounds in pomegranate such as flavonoids, phenolic acids and tannins. Aglycone is usually absorbed from digestive region without damage; however, esters, glycosides and polymers have to be hydrolyzed before absorption (Martos et al., 2010).

Perez-Vicente et al. investigated the *in vitro* digestion of anthocyanins, phenolic compounds and vitamin C of pomegranate juice in their study in 2002. They claimed that phenolic compounds were available during digestion at moderate levels, 29%. However, anthocyanins were degraded or changed into non-red form, 97%, just like vitamin C (Perez-Vicente et al., 2002).

A supplement of 800 mg of pomegranate extract including 330 mg of punicalagins and 22 mg of ellagic acid (EA) were given to 11 healthy volunteers, ellagitannin metabolites, containing EA, urolithin A, hydroxyl urolithin A, urolithin B, urolithin A-glucuronide, and dimethylellagic acid glucuronide in plasma samples between 2 and 24 h after eating, were detected. The antioxidant capacity of plasma increased considerably 32% at 0.5 h postprandial, whereas any effects were not monitored on production of reactive oxygen species. 180 ml of pomegranate juice including 25 mg of EA consumption showed the detection of EA at 32 ng/ml in a 1 h postprandial plasma sample. Taking 180 ml of pomegranate juice concentrate by 18 healthy volunteers showed that EA was present in plasma samples at about 1 h post ingestion. EA can be a useful biomarker, when it was compared with undamaged punicalagin and other ellagitannin metabolites (Basu and Penugonda, 2009).

The bioavailability of pomegranate polyphenols is affected by many factors such as production of juice, analysis methods and variability (Basu and Penugonda, 2009).

2.8 Studies about Changes in Polyphenols, Antioxidant Activity and Vitamin C in Some Fruit and Vegetable Processing and Storage

Processing of fruit and vegetables can affect their antioxidant profiles in different ways. They can have no effect, cause loss of naturally occurring antioxidants, improve antioxidant properties of naturally occurring compounds, form novel compounds having antioxidant activity such as Maillard reaction products and cause interactions among different compounds such as lipids and natural antioxidants, lipids and Maillard reaction products (Nicoli et al., 1999).

Lavelli et al. studied the effect of conventional and new technologies in industrial and pilot plant processing of peach and nectarine purees by means of phenolic content and antioxidant activity. They compared innovative process, operating the pulping step at room temperature and the traditional process of hot pulping. They used DPPH method for antioxidant activity analyses and analyzed proanthocyanidin. They found that industrial methods increased the phenolic content and antioxidant activity, but new methods caused a decrease. The new process decreased the level of cyanidin 3-O-glucoside. The new process was suitable for peach and nectarine which could not be used by conventional technology (Lavelli et al., 2008).

Jimenez et al. determined the effect of canning, freezing and storage for 150 days on antioxidant activity of raw apricot. Canned apricots showed higher ABTS scavenging capacity than raw fruit. Storage of canned or frozen apricots did not create an important difference but antioxidant activity of canned samples was higher than freezed (Jimenez et al., 2008).

Skrede et al. reported changes in anthocyanins and polyphenols during juice processing of highbush blueberries in their study. They researched the effect of processes of blueberries such as milling, depectinization and pasteurization on polyphenols and antioxidant activity. They used pH differential method for determination of total anthocyanin content and analyzed changes in the anthocyanin profile by HPLC. According to results, milling, depectinization and pasteurization caused a significant decrease (Skrede et al., 2000). When blueberries were processed into juice and concentrate, there were important losses of anthocyanins. Milling and depectinization caused losses of anthocyanins and chlorogenic acid and changes in anthocyanin profile. Polyphenolic and anthocyanin loses were lower for concentrating pasteurized juice (Skrede et al. 2000).

In a study by Izquierdo et al., the effects of pasteurization, concentration and freezing at industrial scale on antioxidant capacity and Vitamin C were investigated. They also compared domestic squeezing (hand processing) with commercial squeezing (industrial extraction). Antioxidant capacity was measured by DPPH method and HPLC was used to analyze vitamin C, total and individual phenolics. Commercial squeezing extracted 22% more phenolic than domestic. The freezing caused a sudden decrease in phenolic. For pulp, pasteurization caused degradation of phenolic compounds. Pasteurization, concentration and freezing did not create an important

difference for the total antioxidant capacity of the juice, but decreased 47% in pulp. Commercial squeezing had 25% more of vitamin C than domestic (Izquierdo et al., 2002).

In a study which was published by Capanoglu et al. in 2008, changes in total phenolic, total flavonoid content and antioxidant activity during processing of tomato paste were determined. According to ABTS results, there were no changes, however, for other total antioxidant activity methods, there was a decrease. Total phenolic content increased by 6% and total flavonoid content increased by %31 (Capanoglu et al., 2008a).

Klopotek et al. (2005) studied changes in vitamin C, total phenolics, total anthocyanins and antioxidant capacity during processing strawberries to different products under industrial-like conditions. The effects of juice, nectar, wine and puree production on total phenolic content, total anthocyanin content and hydrophilic antioxidant capacity were measured.

There was a decrease for all parameters in, especially, pasteurization and mashing process steps during making puree, production of nectar and wine. Anthocyanins decreased during fermentation, significantly and the vitamin C content decreased during heat treatment. There was not an important effect of fermentation on total phenolic content (Klopotek et al., 2005).

Khandare et al. reported the effect of processing during production of black carrot juice on antioxidant composition in their study. They researched the effect of pectinase enzyme application before pressing on total phenolic, total flavonoid, total anthocyanin content and antioxidant capacity in laboratory conditions. There were differences according to the amount of enzyme. When the enzyme concentration was 0.2 ml/kg, there was an increase for total phenolics (27%), total flavonoids (46%), total anthocyanins (99%) and antioxidant activity (30%), however when it was 0.25 ml/kg, it caused a significant decrease for all contents and antioxidant activity (Khandare et al., 2011).

Woodward et al. researched changes in anthocyanins during commercial blackcurrant juice processing in 2011. They compared industrial juice production with laboratory conditions. They used UPLC (Ultra Performance Liquid Chromatography) for anthocyanin analysis. Industrial production did not change

anthocyanin content significantly, however, production in laboratory caused losses of 22% of anthocyanins during pasteurization but not in other processes (Woodward et al., 2011).

2.9 Studies about Changes in Polyphenols and Antioxidant Activity in Pomegranate Processing and Storage

Seeram et al. compared antioxidant potency of commonly consumed polyphenol-rich beverages in the United States in their study in 2008. They measured total polyphenol content spectrophotometrically and total antioxidant capacity by ABTS, DPPH, FRAP and ORAC methods. According to results, the highest total phenolic content was belong to pomegranate as 3.8 mg/ml GAE. The antioxidant potency of pomegranate was higher than other beverages at least 20%. The antioxidant potency was from high to low, apple juice, iced tea beverages, orange juice, cranberry juice, acai juice, black cherry juice, blueberry juice, grape juice, red wine and pomegranate juice, respectively (Seeram et al., 2008).

Gil et al. published their study in 2000 as "Antioxidant Activity of Pomegranate Juice and Its Relationship with Phenolic Compostion and Processing" which was the first study about change in antioxidant activity and phenolic content of pomegranate during processing. They also compared antioxidant activity of pomegranate juice with red wine and green tea infusion. They reported that pomegranate juice had three times higher antioxidant activity than red wine and green tea infusion. They compared four different productions: Obtaining juice from arils by hand pressing in laboratory conditions, obtaining juice from frozen and stored (9 months) arils, commercial juice production in a company and commercial concentrate juice production in a company and adding water to obtain juice in laboratory conditions. For antioxidant activity and total phenolic content, commercial juice production and commercial concentrate juice production were equal to each other. For antioxidant activity, obtaining juice from arils and frozen arils were equal to each other, however commercial productions were higher than production from arils in laboratory. The juice which was produced from arils had higher total phenolic and anthocyanin content than the juice which was produced from frozen arils. The juice produced commercially had the highest anthocyanin content, but the concentrated and commercially produced juice which was watered in laboratory had the lowest value.

As a result, they claimed that industrial pomegranate juice production increased total antioxidant activity, total anthocyanin and total phenolic content (Gil et al., 2000).

Turfan et al. researched anthocyanin changes during processing of pomegranate from sacs and whole fruit. The effects of clarification and pasteurization on anthocyanins were evaluated. There was a loss of 4% of anthocyanins in juice produced from sacs and 19% from whole fruit. Pasteurization caused 8-14% loss of anthocyanins for unclarified and 13-19% for clarified. Identification and separation of anthocyanins were carried out by using HPLC. According to HPLC results, cyanidin 3,5-di-O-glucoside was the major anthocyanins, and the others were cyanidin 3-O-glucoside and delphinidin 3-O-glucoside. Their study was the first which reported cyanidin 3,5-di-O-glucoside as the major anthocyanin in pomegranate. They suggested gelatin for cold clarification of pomegranate juice and not to need to use bentonite, kiselsol (Turfan et al., 2011).

Alighourchi et al. characterized anthocyanins of 15 Iranian pomegranates and evaluated their variation after cold storage and pasteurization in laboratory conditions. They determined cyanidin 3,5-di-O-glucoside as the major anthocyanin in pomegranate. They determined anthocyanins of 15 pomegranate varieties, and then 4 of them were separated to analyze the effect of storage time on anthocyanins for unprocessed and pasteurized juices. Anthocyanin analysis was performed by using HPLC. For each anthocyanin, there was a degradation between 23% (cyanidin 3,5-di-O-glucoside) and 83% (delphinidin 3,5-di-O-glucoside) for 10 days at 4^{o}C. Their study showed that cyanidin was the most resistant to degradation than delphinidin and pelargonidin. The anthocyanin content, especially diglucosides, increased after pasteurization and there was also an increase in some anthocyanins after two weeks. During 10 weeks at 4^{o}C, 42.8% of anthocyanins degraded in pasteurized juices (Alighourchi et al., 2008).

Alper et al. researched the effect of processing and pasteurization of pomegranate juice on total phenolic compounds in their study in 2005. They used different clarification techniques such as conventional fining, conventional fining together with FHSV (PVPP) and ultrafiltration. They also compared clarified juices with unclarified juices (control). They claimed conventional fining together with PVPP as the most effective method to remove phenolics. Folin-Ciocalteu method was used for analyzing total phenolic content. Total phenolic reduction in pasteurization was 7.1%

for control, 2.3% for conventional fining, 1.2% for conventional fining with PVPP and 14% for ultrafiltration. Total phenolic reduction in clarification was 20.9% for conventional filling, 31.8% for conventional filling with PVPP and 6.1% for ultrafiltration (Alper et al, 2005).

Mirsaeedghazi et al. investigated the effect of frozen storage at -25^{o}C on anthocyanins and phenolics of pomegranate juice. Storage for 20 days caused loss of 29% of total phenolics and 50% of antioxidant activity. Total anthocyanin content decreased 11% during storage at -25^{o}C for 20 days. There were losses as 3.5% for cyanidin 3,5-di-O-glucoside and 6% for pelargonidin 3,5-di-O-glucoside (Mirsaeedghazi et al., 2011).

2.10 Studies about Total Phenolic Content, Total Flavonoid Content, Tannin Contents and Antioxidant Activity of Different Parts of Pomegranate or Pomegranate Juice Production

Martos et al. researched antioxidant properties of pomegranate juice extraction bagasses in their work. They produced bagasses by extraction arils and peel directly (WFB) and extraction only arils (AB). WFB samples had higher antioxidant capacity, total phenols, total flavonoid and total tannins than AB samples according to all methods. Total phenolic content was 4.62 for AB and 10.05 mg GAE/g sample for WFB. Total flavonoid content was 5.71 for AB and 7.19 mg RE/g samle for WFB (Martos et al., 2011).

Elfalleh et al. researched antioxidant capacities of methanolic extracts of Tunisian pomegranate peel, juice and pulp. For peel and pulp, gallic, ellagic, caffeic and p-coumaric acids were identified. The highest antioxidant activity was in peel with 25.63 mmol TEAC/100 g for FRAP and 22.08 mmol TEAC/100 g for ORAC. Total anthocyanin content was about 73.90 mg CGE/g DW for peel and 39.19 mg CGE/l for juice. Hydrolyzable tannin content 2.69 mg TAE/ml for juice. Antioxidant activity by FRAP was 25.63 mmol TEAC/100g for peel and 7.24 mmol TEAC/l for juice. There were also a high correlation between antioxidant potency and phenolic content for peel extract. Pomegranate peel extracts showed higher antioxidant activity, anthocyanin and hydrolysable tannin content (Elfalleh et al., 2011).

Li et al. researched and compared antioxidant properties of peel and pulp extract in their study in 2006. For extraction pomegranate peel, ethanol, methanol and acetone were used. Pomegranate peel extract had higher antioxidant capacity and all other contents than pulp extract. They suggested pomegranate peel as a supplement and natural antioxidant (Li et al., 2006).

2.11 Studies about the Potential Bioavailability of Some Fruit and Vegetables

There are some studies about using *in vitro* digestion method for determination of potential bioavailability of some fruit and vegetables. McDougall et al. (2005a) researched potential bioavailability of raspberry anthocyanins in their study.

They determined the effect of codigestion of the raspberry extract with common foodstuffs such as bread, breakfast cereal, ice cream and cooked minced beef, and observed the stability and recovery of individual anthocyanins using liquid chromatography-mass spectrometry (LC-MS). The recovery of phenols from digestion was higher than the recovery of anthocyanins. 5% of total anthocyanins entered the IN sample and 70% of them were recovered in the IN and OUT samples. The total phenol content of the IN samples hardly reduced by codigestion with ice-cream or breakfast cereal, however, there was no effect of codigestion with bread or minced beef. The anthocyanin content was not affected or increased by codigestion. According to LC-MS results, all eight anthocyanins found in raspberry were determined in the extract and postgastric samples. For IN and OUT samples, some anthocyanins such as cyanidin 3-O-glucoside was reduced but some such as pelargonidin 3-O-glucoside was increased (McDougall et al., 2005a).

Fazzari et al. researched *in vitro* bioavailability of phenolic compounds from 5 cultivars of frozen sweet cherries by *in vitro* digestion method (2008). After pepsin-HCl digestion, according to the original material, the % recovery of total phenolics increased but the % anthocyanins did not change. After pancreatic digestion and analysis, the total phenolics in IN was about 26-30% and in OUT was 77-101%. The anthocyanin content of IN was 15-21% and OUT was 52-67%. Among five cultivars, Skeena, Lapins and Sweetheart cultivars, which showed higher levels of total phenolics and anthocyanins showed also higher values of these contents in IN and OUT. They also researched the effect of three ripening stages of Bing and Lapins on

bioavailability. Immature cherries showed higher total phenolic content (%) in IN but lower concentration than mature and overmature cherries. It was due to lower total solids content and diffusion rates during dialysis. HPLC analysis of original, IN and OUT samples of Lapins cherries for 3 stages affirmed the results of total phenolics and anthocyanins by spectrophotometric methods (Fazzari et al., 2008).

Wootton-Beard et al. investigated total polyphenol content and total antioxidant capacity of 23 commercially vegetable juices which are significant antioxidant sources by before and after *in vitro* digestion method. Beetroot juice showed higher values than tomato, carrot, mixed vegetable and mixed fruit and vegetable juices.

1369-9500 µmol/l (FRAP), 10.9-90.7% inhibition of $ABTS^{\cdot+}$, 449-3025 µg ferulic acid equivalents/ml (Folin-Ciocalteu) and 57.8-100% inhibition of $DPPH^{\cdot}$ were measured FRAP values for all 23 juices, increased significantly during gastric and intestinal digestion. FRAP value of beetroot juice increased by 2 fold in the gastric phase but a small decrease after intestinal digestion. The same increase in the gastric phase and decrease in the intestinal phase was monitored in DPPH. 18 of the juices increased their ABTS values after the gastric digestion and 12 of the juices continued to increase after the intestinal digestion. For 23 juices, there was an important increase in the gastric digestion and 19 of the juices showed an increase after the intestinal digestion in total polyphenol content (Wootton-Beard et al., 2011).

Bermudez-Soto et al. (2007) investigated polyphenol stability in chokeberry by using *in vitro* gastric and pancreatic digestion. After digestion, they analyzed samples by HPLC-DAD/HPLC–MS to determine the total soluble recovered phenolic content. There was no effect of the gastric digestion on the major phenolic compounds such as anthocyanins, flavan-3-ols, flavonols and caffeic acid derivatives. After the pancreatic digestion, there was a decrease for anthocyanins, flavonols and flavan-3-ols, neochlorogenic acid, chlorogenic acid 43%, 26% and 19%, 28%, 24%, respectively. They claimed that the reason of these losses was not interactions with the digestive enzymes; it was the sensitivity of polyphenols to the alkaline conditions in the intestine (Bermudez-Soto et al., 2007).

Cilla et al. researched total antioxidant capacity, ascorbic acid content and total polyphenols of 8 fruit beverages including grape, orange and apricot, with/without iron and/or zinc and with/without milk. They also investigated the effect of cold

storage (2-4^{o}C) for 135 days and *in vitro* gastrointestinal digestion on total antioxidant capacity. After storage, antioxidant capacity for all samples increased considerably, however ascorbic acid content did not change. At the end of the *in vitro* digestion, antioxidant values of bioaccessible fractions of samples increased 59% and 20% for ORAC and TEAC, respectively, whereas ascorbic acid and polyphenol content decreased 36% and 16%, respectively (Cilla et al., 2011).

McDougall et al., investigated the stability of anthocyanins in red wine under *in vitro* gastrointestinal digestion (2005b). The anthocyanins were resistant to gastric conditions; however, there was a small decrease in total phenol content. At the end of the pancreatic digestion, in IN samples, the total anthocyanins were not recovered completely, but in OUT samples, many of the anthocyanins and phenols were recovered. After the digestion, only 5 of 20 anthocyanins were detected in IN and OUT samples (McDougall, et al., 2005b).

2.12 Studies about the Potential Bioavailability of Pomegranate

Perez-Vicente et al. studied about *in vitro* gastrointestinal digestion of pomegranate juice phenolic compounds, anthocyanins, and vitamin C (2002). They claimed that pomegranate phenolic compounds showed a high amount (29%) during the digestion. Anthocyanins were considerably transformed into non-red forms and anthocyanins and vitamin C were degraded 97% and >95%, respectively. According to HPLC analysis, the total anthocyanin content was 141 mg/l of fresh juice. 6 anthocyanins were determined: delphinidin 3-O-glucoside (5.1%), delphinidin 3,5-di-O-glucoside (18.2%), cyanidin 3-O-glucoside (27.1%), cyanidin 3,5-di-O-glucoside (41%), pelargonidin 3-O-glucoside (5%) and pelargonidin 3,5-di-O-glucoside (3.4%). After the gastric digestion, anthocyanin concentration increased as 10% because of the increase of all anthocyanins, especially, cyanidin and delphinidin glycosides. On the other hand, after the pancreatin bile salt digestion, there was a decrease in anthocyanin concentration due to decrease of all anthocyanins, especially, delphinidin glycosides. The total dialyzed anthocyanin fraction was 2.4% and the nondialyzed fraction was 15.3%. There was not a significant decrease in total phenolic content after the pepsin digestion, however after the pancreatin-bile salt digestion 29% of these compounds were in the dialyzed fraction (Perez-Vicente et al., 2002).

3. MATERIALS AND METHODS

3.1 Materials

Pomegranate samples were taken from industrial scale pasteurized pomegranate nectar manufacturing plant which used pomegranate fruits grown in Karaman. Duplicate samples were taken from 2 different productions. Samples were taken from processing steps and other products, by-products or wastes such as raw material (RM), arils (PA), peels (PP), press cake (PC) and precipitate after clarification (PAC). Processing steps were mashing (PM), pressing (PR), cooling (CO), pasteurization (PAS), enzyme application (EA), clarification (CL), ultrafiltration (UF), concentrate after evaporation (CON), nectar (NE) and pasteurization of nectar (PN), respectively. Totally, 60 samples were obtained and were stored at -80^{o}C until analysis.

For all spectrophotometric and HPLC analysis, Vorteks minishakers-IKA and Magnetic mixer- IKA RH basic 2 were used for mixing and preparation. Glass materials were obtained from ISOLAB.

3.2 Chemicals

For extraction, spectrophotometric analysis, HPLC analysis and potential bioavailability analysis; methanol (≥99.9%), formic acid (≥98%), sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), hydrochloric acid (37%), sulphuric acid ($H_2S_2O_4$, % 95-98), ammonium acetate (NH_4Ac) Copper (II) chloride ($CuCl_2$), methanol (≥99.9%), sodium acetate trihydrate ($CH_3COONa.3H_2O$), potassium persulfate ($K_2S_2O_8$), dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4) and trifluoroacetic acid (99%) were obtained from Merck KGaA (Darmstadt, Germany).

Gallic acid (≥98%), Folin-Ciocalteu phenol reagent, quercetin, catechin (≥98%), neocupraine (Nc), ethanol (≥99.8%), DPPH, TPTZ, ethanol (≥99.8%), potassium disulfite ($K_2O_5S_2$), pepsin, pancreatin, bile salts, acetonitrile (99.8%) were purchased

from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Aluminum chloride ($AlCl_3$), vanillin ($C_8H_8O_3$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Fluka Chemie (Buchs, Switzerland).

Sodium bicarbonate from BDH Chemicals Ltd (Poole, UK), 2,2'-azinobis-3-ethylbenzo-thiazoline-6-sulphonic acid diammonium salt (ABTS) from Applichem GmbH (Darmstadt, Germany), ferric chloride ($FeCl_3$) from Lachema (Czech Republic) and potassium chloride (KCl) from Riedel-de Haen Laborchemikalien GmbH (Hanover, Germany) were purchased.

HPLC standards such as gallic acid (≥99%), (+)-catechin (≥99%), kuromanin chloride (cyanidin 3-*O*-glucoside, ≥96%), pelargonin chloride (pelargonidin 3,5-di-O glucoside), cyanidin chloride (cyanidin 3,5-di-O-glucoside), delphinidin chloride (delphinidin 3-O-glucoside), delphin chloride (delphinidin 3,5-di-O-glucoside) were obtained from Extrasynthese (Genay, France) and the others such as p-coumaric acid (≥98%), chlorogenic acid (≥98%), quercetin-3-O-galactoside (≥98%), ferulic acid (≥98%) were purchased from Fluka Chemie (Buchs, Switzerland) and quercetin-3-β-D-glucoside (≥98%) and callistephin chloride (pelargonidin 3-O-glucoside) were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Water used for all analysis was distilled and purified with the water purification system (TKA GenPure, Germany).

3.3 Method

Moisture analysis was performed for all pasteurized pomegranate nectar processing samples. Total phenolic content, total flavonoid content, total anthocyanin content, total tannin content, total antioxidant activity (CUPRAC, DPPH, FRAP, ABTS), and polymeric color (%) analysis were carried out by using spectrophotometric methods. HPLC analysis of major phenolic compounds and anthocyanins was performed for each sample. Total phenolic content analysis, total antioxidant activity analysis by DPPH, total anthocyanin analysis by pH differential method and HPLC analysis of major phenolic compounds and anthocyanins were also performed for bioavailability samples of peel, arils, concentrate, press cake, precipitate after clarification and pasteurized nectar.

3.3.1 Pasteurized pomegranate nectar manufacturing and sampling

After whole pomegranate receival, pomegranates were washed with water and foreign materials such as the stones and herbs were separated. Then they were washed with distilled water and selected. The peels of pomegranate were separated by peeling. The arils were placed in mashing tanks and pectinase enzyme was added. After mashing, they were pressed and press cake was obtained. They were stored after cooling till pasteurization. Pasteurization was performed at 90-95^{o}C and pasteurized samples were cooled to 50-55^{o}C. Then pectinase was added in enzyme application step. To clarify the juice bentonite and gelatin were added. At the end of the clarification, precipitate was separated. After clarification, ultrafiltration was performed and the ultrafiltrated juice was evaporated to obtain concentrated juice. By addition of glucose and water, nectar was produced and it was pasteurized. The block diagram for production of pasteurized pomegranate juice is shown in Figure 3.1 in which pink color steps are the sampling points and the others are white.

3.3.2 Sample preparation

Samples were milled under liquid nitrogen using grinder (IKA, Germany). Ground samples were stored at -80^{o}C.

3.3.3 Moisture analysis

To express the results in dry matter basis, moisture content analysis was performed as described in Turkish Standards (TS 1129, 1998). 2 g of each sample were weighed and placed in disposable aluminum containers. Drying was performed by vacuum oven (Gallenkamp, UK) at 70^{o}C for 6 hours under 600 kPa pressure.

3.3.4 Extraction

Extraction procedure was adapted from Bino et al. (2005). For all steps 1g of sample, except for the peels (0.5 g), was weighed under liquid nitrogen. They were placed in test tubes and 5 ml of 75% methanol: water solution with 0.1% formic acid was added to each. Then samples were sonicated for 15 minutes in ultrasonic bath (Ultrasonic Cleaner-VWR). After sonication they were centrifuged (Universal 32, Tuttlingen, Germany) at 4000 rpm under 4^{o}C and the centrifugates were collected.

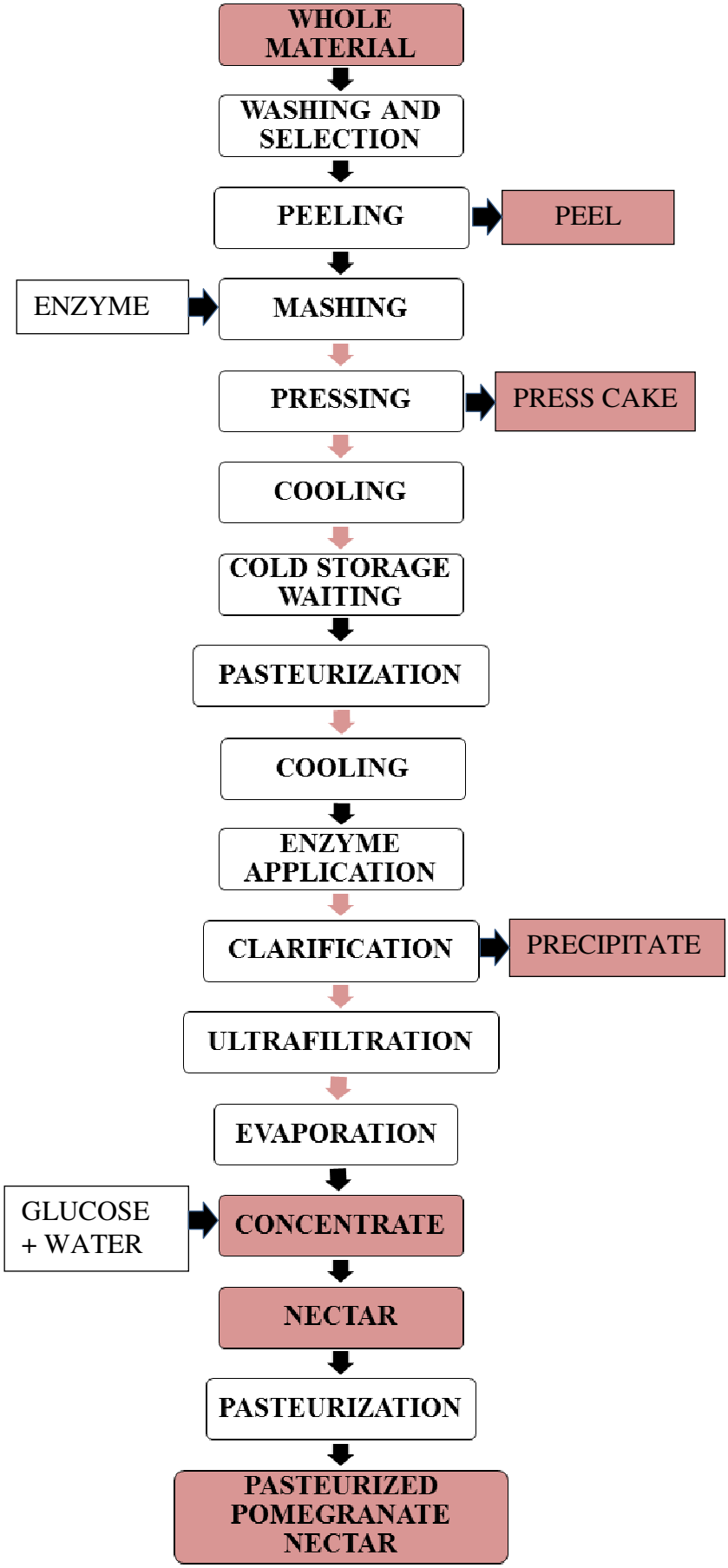


Figure 3.1: Production steps for pasteurized pomegranate juice.

This extraction procedure was repeated 4 times with fresh solvent until 20 ml of solvent is used. Sample extracts were stored at -20^{o}C until analysis.

3.3.5 Total phenolic content

Folin Ciocalteu method is commonly used to measure total phenolic content, however it does not only measure phenolic compounds but several non-phenolic compounds such as some nitrogen containing compounds, vitamin C and Cu(I) which can react with this agent.

Folin-Ciocalteu method which was used by Velioglu et al. (1998) was modified to measure the total phenolic content. The extract (100µl) was vortexed by vorteks minishakers (IKA, Germany) with 750 µl of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and then 750 µl sodium carbonate (60 g/l) was added to the mixture. After incubating for 90 min at room temperature by protecting from light, absorbance was measured at 725 nm using a UV-Visible spectrophotometer (Shimadzu UV-1700 UV-Vis).

The calibration curve was prepared by using gallic acid standard solutions. The results were expressed in gallic acid equivalents (GAE) per 100 g of dry weight (DW) and reported as mean value ± SD.

3.3.6 Total flavonoid content

The method used by Martos et al. (2011) was adapted to measure the total flavonoid content. 1 ml of extract was mixed with 0.3 ml $NaNO_2$ (5%). After 5 minutes, 0.3 ml $AlCl_3$ was added and in the sixth minute, 2 ml NaOH was added for neutralizing. Finally, 2.4 ml of water was added to mixture and it was mixed. The absorbance was read using spectrophotometer (Shimadzu UV-1700 UV-Vis) at 510 nm.

The calibration curve was prepared by using quercetin standard solutions. The results were expressed as quercetin equivalents (QE) per 100 g of DW and reported as mean value ± SD.

3.3.7 Total anthocyanin content

pH differential method is a common, basic and rapid spectrophotometric method which is used by food technologists to evaluate the quality of fruit and vegetables. It is used to measure total monomeric anthocyanin content by the principle which is

based on change in absorbance of the anthocyanin at two different pH values of 1.0 and 4.5 at different wavelengths (λ) of 520 and 700 nm. Monomeric anthocyanins cause a colored form at pH 1.0 and colorless form at 4.5. The difference in absorbance at 520 nm for the pigment is proportional to the concentration of pigment. Anthocyanins which are degraded in the polymeric form are resistant to color change, so polymerized anthocyanin pigments cannot be determined due to absorption at pH 4.5 and 1.0. The absorbance has to be measured at the $\lambda_{vis\text{-}max}$ of the pigment solution and molar extinction coefficient and molecular weight of major anthocyanin in the matrix (Lee et al., 2005). In this study the anthocyanin content of pomegranate juice was expressed as cyanidin-3-glucoside (cyn-3-gly).

Total anthocyanin content was measured by the method, as described by Kar et al. (2011) in pomegranate juices. Two buffer solutions, 0.025 M KCl and 0.4 M $CH_3COONa.3H_20$ were prepared by using distilled water. KCl and $CH_3COONa.3H_20$ solutions were concentrated to pH 1.0 and pH 4.5, respectively, by using 37% HCl. Extracts were diluted and mixed with these solutions. After 15 minutes, the absorbance reading was performed at 520 and 700 nm. Analyses were carried out in triplicate for each sample. The difference in absorbance was expressed by using equation 3.1.

$$A = (A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5} \quad \textbf{(3.1)}$$

A = Difference in absorbance

A_{520} = Absorbance at 520 nm

A_{700} = Absorbance at 700 nm

The total anthocyanin content was evaluated and expressed by using equation 3.2.

$$\text{mg cyn-3-gly equivalents/100 g} = \frac{A * MW * DF * 100 * 1000 * 20}{e * 1 * 1000 * sampleweight} \quad \textbf{(3.2)}$$

MW: Molecular Weight = 449.2 g/mol for cyn-3-gly

DF: Dilution Factor

e: Molar Extinction Coefficient = 26900 in $Lmol^{-1} * cm^{-1}$ for cyn-3-gl

3.3.8 Total tannin content

Due to deficiency of useful methods for isolation and structure explanation, chemical research of tannins started later. There is no single method for analysis of all tannins,

especially, for higly-polymerised tannins which are difficult to analyze. Several studies in the literature analyze low molecular weight tannins. Tannins can also react with ferrum chloride and Folin-Ciocalteu. For quantification of tannins color reactions are usually preferred which are based on spectrophotometric measurements. When proanthocyanidins react with mineral acids or vanillin-HCl, they cause red reaction products. The reaction between dimethylaminobenzaldehyde and proanthocyanidins give blue reaction products (Serrano et al., 2009).

Total tannin content was analyzed by vanillin method based on reaction between proanthocyanidin and vanillin spectrophotometrically (Kar et al., 2011). Two mililiters of vanillin (1% in 7 M H_2SO_4, prepared in an ice bath) was added to 1 ml of extract and mixed. After 15 min, the absorbance of the solution was read at 500 nm.

Standard curve was prepared with catechin in 1% formic acid containing 75% metanol-water solution. The results were expressed as catechin equivalents (CE) per 100 g of DW and reported as mean value ± SD.

3.3.9 Polymeric color (%) analysis

Polymeric color percentage (Color Due Tannin) is used to express the degree of polymerization. Bisulfite solutions are used to analyze polymeric color (%). Anthocyanin pigments react with bisulfite to form a colorless sulfonic acid adduct as shown in Figure 3.2.

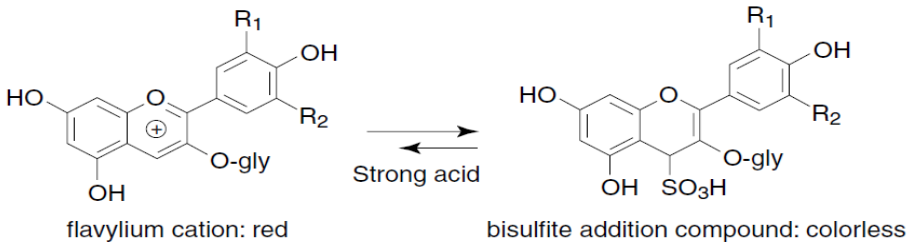


Figure 3.2: Colorless anthocyanin-sulfonic acid adducts (Giusti & Wrolstad, 2001).

Anthocyanin-tannin complexes are resistant to bleaching by bisulfite, however, the bleaching reaction of monomeric anthocyanins complete very quickly. The measurement of absorbance of the bisulfite-added sample at 420 nm is obtained as an index for browning. Color density is expressed as the sum of absorbances at the λvis-max and at 420 nm. The percentage of the color is calculated by using polymerized

color and color density (Giusti and Wrolstad, 2001). Distilled water samples are used as controls.

Polymeric color (%) analysis was calculated for each sample, except for nectar, as described by Giusti and Wrolstad et al. (2001). Two tubes from 2.8 ml of extract were prepared without dilution or using dilution rate used for preparing KCl solutions in pH diferential method. 0.2 ml of distilled water was added to one of them and 0.2 ml of potassium metabisulfite (potassium disulfite, $K_2S_2O_5$, 1 g in 5 ml of distilled water) was added to another. After mixing and waiting for 15 minutes, absorbance values were measured for each tube against distilled water at 420, 520 and 700 nm. Analyses were performed in duplicate for each sample. Polymeric color and color density was calculated as shown in equation 3.3 and 3.4, respectively. Polymeric color (%) was obtained by dividing polymeric color to color density as in equation 3.5.

$$\text{Color density} = [(A_{420\,nm} - A_{700nm}) + (A_{520\,nm} - A_{700\,nm})] \times DF \quad \textbf{(3.3)}$$

$$\text{Polymeric color} = [(A_{420\,nm} - A_{700\,nm}) + (A_{520\,nm} - A_{700\,nm})] \times DF \quad \textbf{(3.4)}$$

$$\%\ \text{Polymeric color} = (\text{Polymeric Color/Color Density}) \times 100 \quad \textbf{(3.5)}$$

3.3.10 Total antioxidant activity analysis

The antioxidant activity methods commonly used in analyses are 2,2-azino-bis-3 ethylbenzthiazoline-6-sulphonic acid (ABTS), 2,2 diphenyl-1-picrylhydrazyl (DPPH), Cupric Reducing Antioxidant Capacity (CUPRAC), Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Ability of Plasma (FRAP) assays. Most of these analyses have the same principle. An artificial colored radical or redox-active compound is produced and the sample scavenges the radical or reduces the redox-active compound. The capacity of this reaction is observed by using spectrophotometer and expressed by a convenient standard such as Trolox Equivalent Antioxidant Capacity (TEAC) or Vitamin C Equivalent Antioxidant Capacity (VCEAC). The methods are divided into three groups: Based on an electron transfer, based on a hydrogen atom transfer and including reduction of a colored oxidant. ORAC is the method which includes a hydrogen atom transfer and finds chemical change in a fluorescent molecule caused by a free radical attack. ABTS,

DPPH and FRAP involves reduction reactions. The ABTS method is based on the production of a blue/green $ABTS^{\bullet+}$ that is useful for hydrophilic and lipophilic antioxidant systems and the DPPH method is based on the reduction of the purple 2,2-diphenyl-1-picryl hydrazine which is dissolved in organic media, so it is useful for hydrophobic systems. On the other hand, there are no free radicals in the FRAP method, but the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) is observed. The antioxidant capacity measurements of foods are dependent on the method used (Floegel et al., 2011).

3.3.10.1 Cupric reducing antioxidant capacity (CUPRAC) analysis method

CUPRAC method is used to measure total antioxidant activity from the vegetable matrix. Apak et al. aimed to establish a new method for measuring total antioxidant including dietary polyphenols, vitamin C and E by using copper (II)- neocuproine [Cu(II)-Nc] reagent. Due to measurement of copper (II) ion reducing ability of polyphenols, they named the method as CUPRAC. They claimed that it was useful than FRAP because the chemistry of copper (II) had faster kinetics. CUPRAC method includes a mix of antioxidant solution, copper (II) chloride solution, neocuproine alcoholic solution, and an ammonium acetate aqueous buffer solution at pH 7.0 (Apak et al., 2004). Antioxidant analysis by CUPRAC is based on reducing of Cu(II) to Cu(I) and gives the power of antioxidant for reducing Cu(II). Cu(I) gives absorbance with neocuproine (2,9- dimetil-1,10- fenantrolin) as showed in Figure 3.3 at 450 nm. Copper is more advantageous than ferrous for antioxidant analysis (Prior et al., 2005).

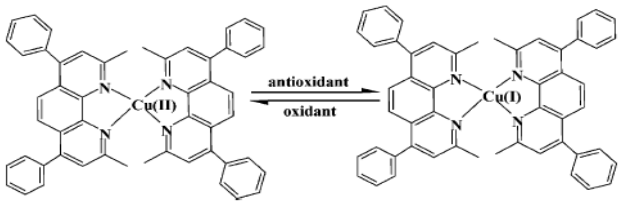


Figure 3.3: The formation of Cu(I) complex (Huang et al., 2005).

For CUPRAC analysis, to a test tube 100 µl sample, 1 ml $CuCl_2$ solution ($1.0x10^{-2}$ M), 1 ml neocuproine alcoholic solution ($7.5x10^{-3}$ M) and 1 ml NH_4Ac buffer solution at pH 7.0 were added. To make the volume 4.1, finally 1 ml water added and mixed well. Absorbance against a reagent blank was measured after 1 h (Apak et al.,

2006). Standard curve was prepared with Trolox in 75% methanol-water containing 1% formic acid and the results were expressed as Trolox equivalents (TEAC) per 100 g of DW and reported as mean value ± SD.

3.3.10.2 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The DPPH method measures the capacity of the extract to give hydrogen to DPPH radical and bleach the DPPH solution (Martos et al., 2011). The structure of DPPH radical is shown in Figure 3.4. It is the stable nitrogen radical giving dark purple color (Prior et al., 2005).

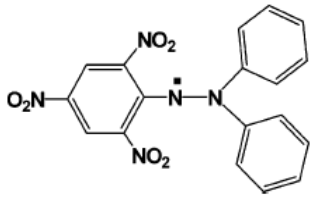


Figure 3.4: DPPH· (2,2 diphenyl -1-picrylhydrazyl) (Prior et al., 2005).

In the presence of antioxidants, the dark purple color of DPPH radical changes into yellow color. The higher bleaching reaction means the higher antioxidant activity. The DPPH scavenging data claims that the extract has the ability of scavenging free radicals and protecting from initiation and propagation, which are the chain reactions. This is useful for the preservation of foods, drugs and cosmetics by means of retarding lipid oxidation and deterioration (Martos et al., 2011).

DPPH is very fast and simple method, however, one of disadvantages is to precipitate many proteins and conclude at different reaction times according to number of hydroxyl (-OH) groups (Vattem ve Shetty, 2007).

Total antioxidant activity was measured by DPPH method adapted from Martos et al. (2011). 0.1 mM of DPPH solution was prepared by using 100% methanol. 100 µl of extract was mixed with 2 ml of DPPH solution. After keeping 30 min in the dark, absorbance was read at 517 nm.

Standard curve was prepared with Trolox in 75% met-water involving 1% formic acid and the results were expressed as Trolox equivalents (TEAC) per 100 g of DW and reported as mean value ± SD.

3.3.10.3 Ferric reducing ability of plasma (FRAP) analysis method

The FRAP method measures the ferric reducing ability of plasma. At low pH 3.6, the reduction of ferric to ferrous ion generates a colored ferrous-tripyridyltriazine complex. The concentration of ferrous ion helps to determine concentration in the test reaction at 593 nm (Benzie et al., 1996).

FRAP has electron-transfer reaction mechanism. $Fe(III)(TPTZ)_2Cl_3$ complex acts as an oxidant. FRAP reagent was obtained by mixing TPTZ (2,4,6- tripridil-s-triazin), CH_3COONa buffer solution and $FeCl_3.H_20$. Dark blue colored $[Fe(II)(TPTZ)_2]^{+2}$ complex gives the maximum absorbance at 593 nm as described in Figure 3.5 (Huang et al., 2005). The intensity of the color is proportional to the antioxidant activity.

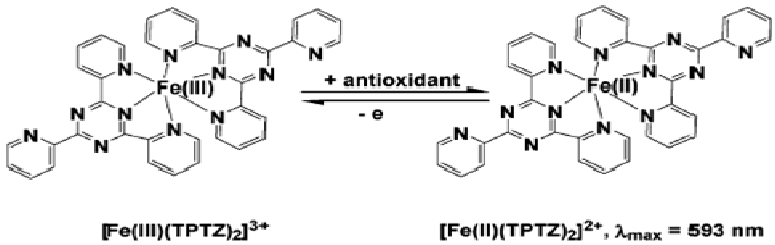


Figure 3.5: The formation of $[Fe(II)(TPTZ)_2]^{+2}$ complex (Huang et al., 2005).

Total antioxidant activity was measured by FRAP method as explained by Deighton et al. (2000) and Benzie et al. (1996). 20mM $FeCl_3.H_20$, 10mM TPTZ (2,4,6-tripyridyl-s-triazine) and 0.3 M CH_3COONa buffer solution at pH 6.0 were prepared. Fresh FRAP reagent was prepared by mixing these solutions in 10:1:1: ratio for CH3COONa, TPTZ and $FeCl_3.H_20$, respectively. 100 µl of extract was mixed with 900 µl of FRAP reagent and after 4 min, absorbance was read at 593 nm.

Standard curve was prepared with Trolox in 75% methanol-water involving 1% formic acid and the results were expressed as Trolox equivalents (TEAC) per 100 g of DW and reported as mean value ± SD.

3.3.10.4 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) analysis method

Antioxidant activity analysis by ABTS method is based on the formation of $ABTS^{\bullet-}$ oxidant by the result of the oxidation of $ABTS^{-2}$ with persulphate and reading

maximum absorbance at 734 nm (Huang et al., 2005). ABTS method has the similar principle with that of DPPH. ABTS reacts with hydroxyl, peroxyl, alkoxyl ve inorganic radicals and forms stable ABTS radical cation. Antioxidant or reactive oxygen species catch free radicals and prevents ABTS cation radical formation. This radical catching activity is measured spectrophotometrically (Vattem and Shetty, 2007). The generation of ABTS $^{-2}$ ion is shown in Figure 3.6.

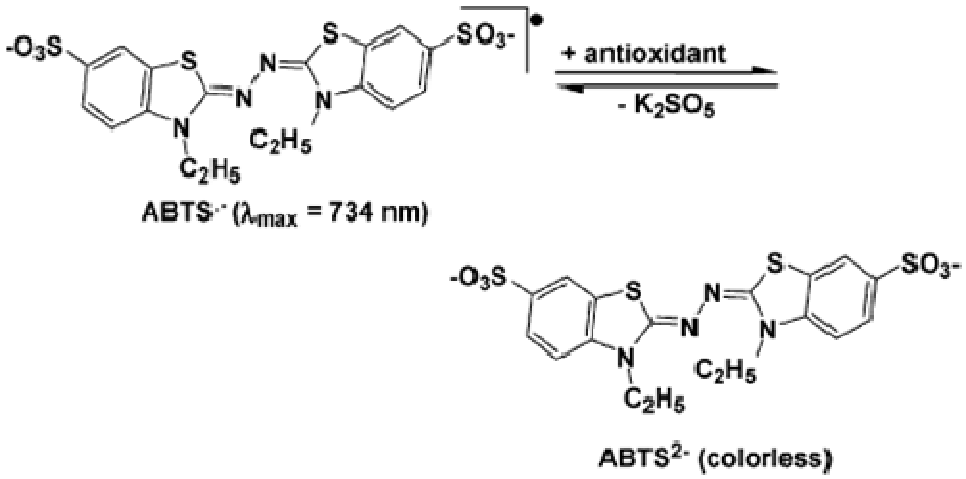


Figure 3.6: The generation of ABTS $^{-2}$ ion (Huang et al., 2005).

Analysis of antioxidant activity was performed as described by Miller and Rice-Evans (1997). ABTS reagent and potassium persulfate solutions ($K_2S_2O_8$) were prepared by dissolving 200 mg of ABTS in 200 ml water and 38 mg of $K_2S_2O_5$ in 2 ml. These solutions were mixed and left for one night to form the radical.

0.05 M buffer solution (KPi) at pH 8.0 was prepared by mixing 0.05 M potassium dihydrogen phosphate (KH_2PO_4) and 0.05 M dipotassium hydrogen phosphate (K_2HPO_4). ABTS reagent mixture was prepared by mixing ABTS and KPi solution and measuring absorbance at 0.9 ± 0.2.

100 µl of extract was taken and 1 ml of prepared ABTS reagent mixture was added and mixed for 15 seconds. After waiting for 45 seconds, absorbance was read at 734 nm against water blank.

Standard curve was prepared with Trolox in 75% met-water involving 1% formic acid and the results were expressed as Trolox equivalents (TEAC) per 100 g of dry weight and reported as mean value ± SD.

3.3.11 *In Vitro* digestion method for potential bioavailability

To evaluate bioavailability of any phytochemical, information is necessary about its absorption, metabolism, distribution in tissue and organs and excretion. Studies about animals and humans are usually not preferred due to be complicated, expensive and also ethical restrictions (Guven et al., 2010). *In vitro* digestion method is a basic and fast method that can evaluate the potential stability of phytochemicals from fresh, extracted and processed foods (McDougall et al., 2005a).

Reproduction of transport and metabolic mechanism cannot be effectively achieved, but by mimicking the gastrointestinal digestion, the potential bioavailability of compounds can be determined in studies. Besides determination of bioavailability, this system helps to compare multiple samples and obtain information about the effect of food matrices on the recovery of compounds. First *in vitro* digestion method was performed by Miller et al. in 1981. They compared the iron content of IN samples with serum iron bioavailability *in vivo*. Then, this method was adapted and used to determine the potential bioavailability of anthocyanins and other phenolic compounds in fruits in some studies by Gil-Izquierdo et al. for orange juice (2001), Perez-Vicente et al for pomegranate juice (2002), McDougall et al. for raspberry and red wine (2005a, 2005b), Fazzari et al. for frozen cherries (2008), respectively (Fazzari et al, 2008).

Bioavailability procedure was carried out as decribed by McDougall et al. (2005a). The method consists of two parts: gastric conditions and small intestine conditions. Gastric and small intestine conditions were applied by pepsin-HCl digestion for 2 h at 37^{o}C and bile salts-pancreatin for 2 h at 37^{o}C, respectively. 5 g of ground sample was weighed in a glass beaker by using liquid nitrogen and 20 ml of distilled water was added and mixed. 315 units/ml pepsin was used to prepare pepsin solution and 1.5 ml of this solution was added to sample in distilled water. pH of sample solution was adjusted to 1.7 by using 5 M HCl and incubated for 2 h with shaking at 100 rpm in a heated water bath at 37^{o}C. After 2 h, postgastric digestion was completed and 2 ml of the solution was removed and stored in eppendorf tubes at -20^{o}C for further analysis. 4.5 ml of 4 mg/ml pancreatin and 4.5 ml of 25 mg/ml bile salts mixture was added. A piece of cellulose dialysis tubing (molecular mass cutoff, 12 kDa) which was washed with distilled water before using was prepared, the bottom of the tubing was linked tightly and 20 ml of 1 M $NaHCO_3$ was added for neutralizing the

sample's titratable acidity. The beaker was closed by using Parafilm and placed again in the heated water bath at 37°C for 2 h. After incubation at small intestine conditions, the solution outside the tubing was taken in eppendorf tubes as OUT sample which was the sample that did not enter the serum. The solution entering the dialysis tubing was taken in eppendorf tubes as IN sample which was in the serum. IN and OUT samples were centrifuged at 16000 rpm for 20 min in a microfuge if necessary. The centrifugates were stored at -20°C for analysis.

Total phenolic content analysis by Folin-Ciocalteu method, total antioxidant activity analysis by DPPH method and total anthocyanin analysis by pH differential method were performed for 6 samples which were either products, by-products or wastes such as peel, arils, concentrate, press cake, precipitate after clarification and pasteurized nectar. The analyses were carried out for PG, IN and OUT for each sample in triplicate. Major phenolic compound and anthocyanin analysis by using HPLC-PDA analysis were also performed for bioavailability samples.

3.3.12 HPLC-PDA analysis of major phenolic compounds and anthocyanins

Major phenolic compounds were analyzed by using the method as described by Capanoglu et al. (2008b). Standard calibration curves were prepared by using catechin, gallic acid, quercetin-3-β-D-glucoside (q-3-BDg), quercetin-3-galactoside (q-3-g), p-coumaric acid, neochlorogenic acid, ferulic acid, kuromanin chloride (cyanidin 3-O-glucoside, cyn 3-O-glu), cyanin chloride (cyanidin 3,5-di-O-glucoside, cyn 3,5-dOg), delphnidin chloride (delphinidin 3-O-glucoside, del 3-O-glu), delphin chloride (delphinidin 3,5-di-O-glucoside, del 3,5-dOg), callistephin chloride (pelargonidin 3-O-glucoside, pel 3-O-glu) and pelargonin chloride (pelargonidin 3,5-di-O-glucoside, pel 3,5-dOg). Methanolic extracts were filtered through a 0.45-µm membrane filter and 1 ml of the filtered extract was placed into vials and analyzed in a Waters 2695 HPLC system with PDA (Waters 2996), for each sample. Luna C18 column (Phenomenex) was used as the stationary phase.

The mobile phase was including solvent A, Milli-Q water with 0.1% (v/v) TFA and solvent B, acetonitrile with 0.1% (v/v) TFA. A linear gradient was used as follows: at 0 min, 95% solvent A and %5 solvent B; at 45 min, 65% solvent A and 35% solvent B; at 47 min, 25% solvent A and 75% solvent B; and at 54 min returns to initial conditions. The flow rate was 1 ml/min. Detections were done at 280, 312, 360, and

512 nm wavelengths. Identification was based on the retention times and characteristic UV spectra and quantification was done by external standard curves. All analyses were performed in triplicate.

3.4 Statistical Analysis

The results were analyzed statistically by using one way analysis of variance (ANOVA), Duncan's New Multiple Range Test and Statistical Package for the Social Sciences (SPSS) 16.0 version at significant level as 0.05. The differences between all samples; all processing steps, the product and raw material; and raw material, arils, the product and waste products were evaluated statistically. Duncan's New Multiple Range Test was applied to mean values to observe the difference between contents, antioxidant activity or amount of phenolics and anthocyanins ($p<0.05$). All of the comparisons and statistical evaluation by SPSS are showed in APPENDIX A. To observe the relation between methods, basic regresyon analysis was performed and significant relations were determined statistically.

4. RESULTS AND DISCUSSION

All of the results were expressed by using standards and reported as mg equivalents /100 g dry weight (DW). Each analysis was performed in triplicate for each sample. Statitical evaluation and ANOVA tables of all analysis by SPSS were presented in Table A.1-A.10.

4.1 Total Phenolic Content

Results for total phenolic content analysis were expressed as mg gallic acid equivalents (GAE)/100 g DW for each sample. The standard calibration curve of gallic acid shown in Figure 4.1 was prepared between 0.02-0.2 mg/ml and the equation obtained from the curve was used to calculate the absorbance values of the samples measured by UV-Vis spectrophotometer.

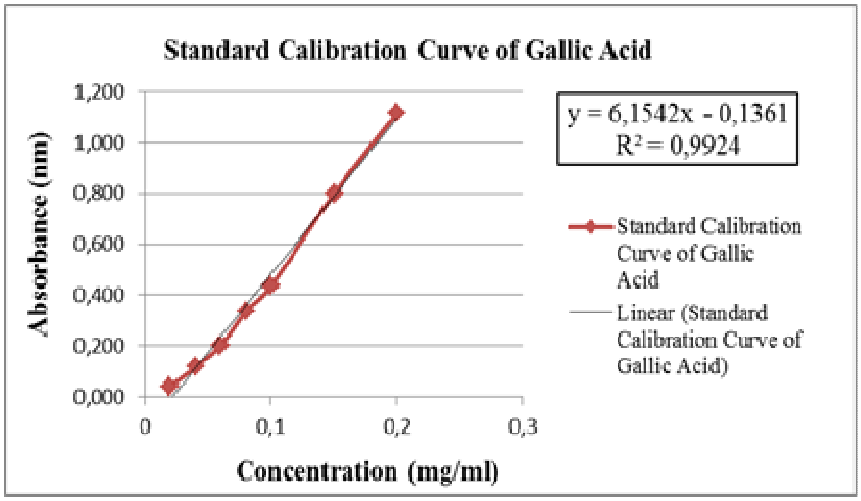


Figure 4.1: Standard calibration curve of gallic acid.

Results and statistical evaluation of total phenolic content analysis are showed in Table 4.1. According to this table pomegranate peel showed the highest total phenolic content value and pasteurized nectar the lowest. Pasteurization of nectar did not change the content. Raw material showed higher value than arils due to including peel.

Table 4.1: Total phenolic contents for all samples.

Sample	mg GAE/100 g DW
Peel (PP)	18029.17 ± 1568.87 a
Raw material (RM)	11161.45 ± 3245.81 b
Arils (PA)	5477.35 ± 972.95 c
Mashing (PM)	5176.76 ± 464.35 cd
Pressing (PR)	4924.08 ± 521.09 cde
Press cake (PC)	3171.29 ± 390.91 e
Cooling (CO)	5465.87 ± 675.13 c
Pasteurization (PAS)	4912.00 ± 49.87 cde
Enzyme application (EA)	4939.42 ± 808.43 cde
Clarification (CL)	4530.75 ± 1113.44 cde
Precipitate after clarification (PAC)	3318.80 ± 501.29 de
Ultrafiltration (UF)	4088.13 ± 734.63 cde
Concentrate (CON)	3631.30 ± 217.27cde
Nectar (NE)	1081.53 ± 233.14f
Pasteurized Nectar (PN)	1005.31 ± 115.50f

As shown in Figure 4.2, during production of pasteurized pomegranate nectar, there was a general decreasing trend; especially highest reductions were obtained during mashing and nectar production from concentrate. These severe decreases were probably due to discarding of pomegranate peel before mashing and addition of glucose during nectar production. On the other hand, there was no significant change in total phenolic content from mashing to evaporation ($p>0.05$), so these process steps did not create a significant difference in total phenolic content.

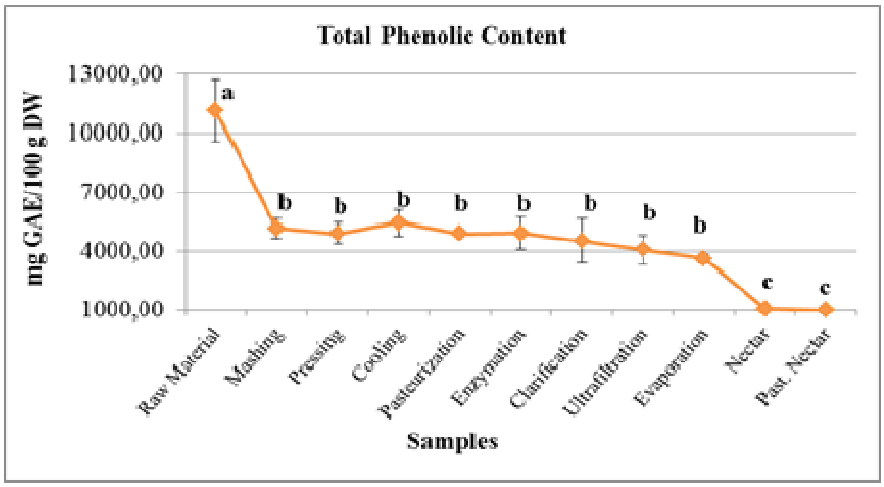


Figure 4.2: Change in total phenolic content during pomegranate nectar production.

Raw material and the product are also assessed besides waste products and arils as seen in Figure 4.3. Press cake and precipitate showed the same level of phenolics as arils or the product, but lower than that of peel and raw material.

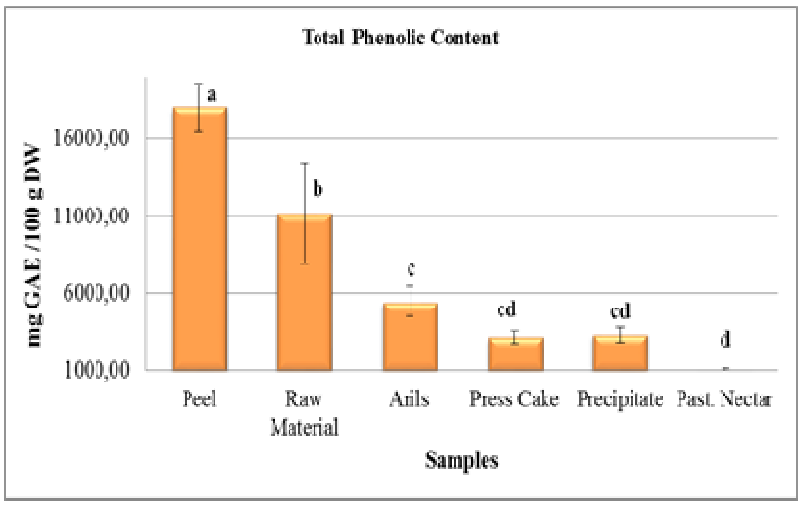


Figure 4.3: Total phenolic contents of raw materials, waste products and the product.

Martos et al. (2011) obtained total phenolic content of pomegranate juice arils bagasse and whole fruit bagasse (4.62 ± 0.74, 10.05 ± 1.20 mg GAE/g sample, respectively). In that study, total phenolic content of press cake was calculated as 31.71 ± 3.90 mg GAE/g DW, which was greater than 15.41 ± 1.80 mg GAE/g for the sample. Ardekani et al. (2011) found total phenolic content for the peel extract in the range of 98.24 ± 4.81 - 226.56 ± 18.98 mg GAE/g dry extract. On the other hand, Li et al. (2006) found 249 ± 17.2 mg tannic acid/g fresh extract and Nasr et al. (1996) had 216 ± 7.3 mg GAE/g DW. In this study, total phenolic content of peel was found as 180.29 ± 15.68 mg GAE/g DW and 55.34 ± 5.63 mg GAE/g FW which was consistent with Ardekani et al. and closer to Nasr et al. and lower than Li et al. due to the difference in the used standard. Karadeniz et al. (2005) compared selected fruit and vegetables grown in Turkey and they reported total phenolic content of pomegranate as 240.8 ± 38.9 mg CE/100 g FW, however, in this study it was found as 2955.2 mg/100 g FW. Karadeniz et al. purchased pomegranate from local markets in Turkey and prepared standard calibration curve by catechin which may be the reason for the difference.

Alper et al. (2005) found total phenolic reduction in clarification during production of pomegranate juice from raw material as 20.9% for conventional fining; however, in this study the reduction in clarification was about 60%. This difference may be due to difference in raw material and production methods. Klopotek et al. (2005) claimed that during the production of juice and nectar of strawberry, the highest losses for phenolic content were in mashing and production of nectar similar to the findings obtained in this study.

The phenolic content can be different according to several factors such as the cultivar, growing region, climate, maturity, cultivation practice, storage conditions, method used to produce the juice and performed in the analysis (Martos et al., 2011).

4.2 Total Flavonoid Content

Standard calibration curve of quercetin was prepared (Figure 4.4). Total flavonoid contents were expressed as mg quercetin equivalents (QE)/100 g DW for each sample. The standard calibration curve prepared in between 0.005-0.4 mg/ml and the equation was used to evaluate the absorbance values of the samples measured by UV-Vis spectrophotometer.

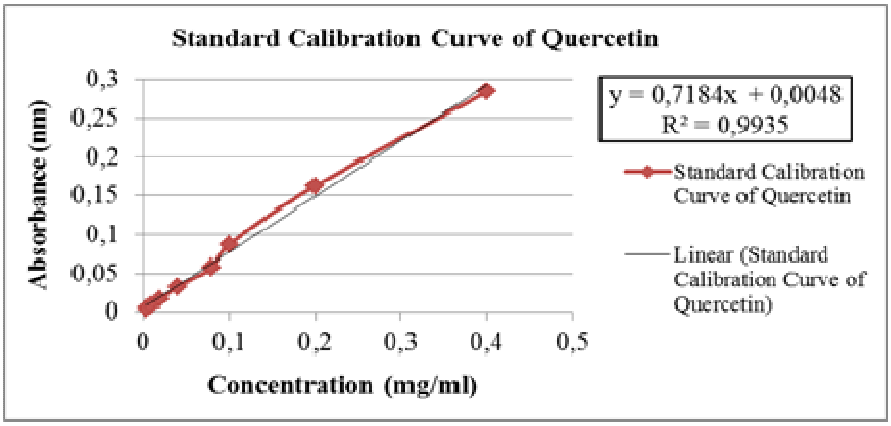


Figure 4.4: Standard calibration curve of quercetin.

Pomegranate peel showed the highest total flavonoid content value (23005.88 mg QE/100 g DW) and nectar the lowest (666.12 mg QE/100 g DW); however, there was no significant difference between pasteurization, nectar production and pasteurization of nectar ($p>0.05$).

Raw material showed higher value than arils as reported in total phenolic content because of consisting pomegranate peel which is the source for phenolics and flavonoids.

The flavonoid content of fruits also depends on many factors similar to total phenolic content such as properties of the fruit; growing and climate conditions and processing and storage conditions (Martos et al., 2011). Results and statistical evaluation for total flavonoid content analysis of all samples are shown in Table 4.2.

Table 4.2: Total flavonoid contents for all samples.

Sample	mg QE/100 g DW
PP	23005.88 ± 2542.74 a
RM	14127.05 ± 3691.79 b
PA	6423.42 ± 2682.70 c
PM	4852.99 ± 1586.84 c
PR	4273.44 ± 1599.04 c
PC	3739.24 ± 802.70 c
CO	4430.95 ± 1654.21 c
PAS	1055.00 ± 122.70 d
EA	5626.03 ± 938.01 c
CL	4602.62 ± 931.15 c
PAC	3637.45 ± 507.95 c
UF	3837.02 ± 508.91 c
CON	3796.06 ± 407.18 c
NE	666.12 ± 457.80 d
PN	1023.88 ± 314.47 d

Change in total flavonoid content during production of pasteurized nectar is shown in Figure 4.5. There was an important decrease from raw material to mashing due to loss of flavonoids in peel, but no change was observed from mashing to pasteurization. After a decrease in pasteurization, by the enzyme application increasing trend was observed and stability continued until nectar production. Production of nectar by adding glucose caused a significant reduction ($p<0.05$).

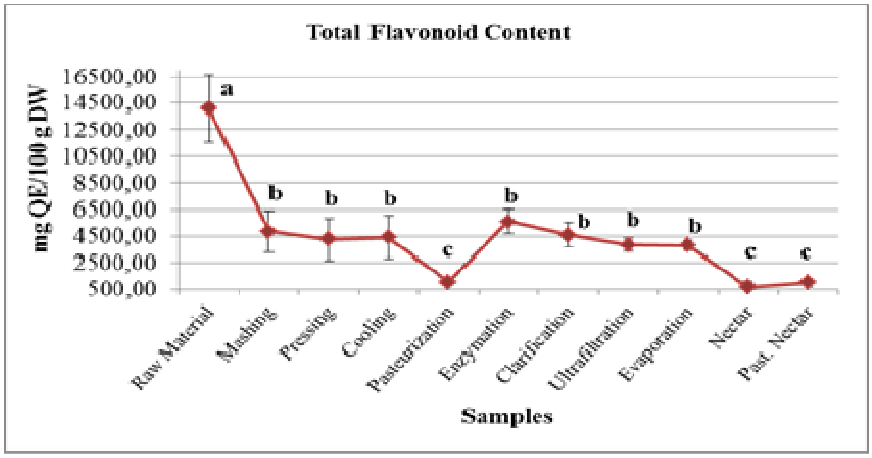


Figure 4.5: Change in total flavonoid content during pomegranate nectar production.

The evaluation of raw material and the product with waste products and arils was presented in Figure 4.6. Press cake and precipitate after clarification showed the same flavonoid content with arils or the product.

They were significantly lower than peel and raw material ($p<0.05$). The results were consistent with total phenolic content.

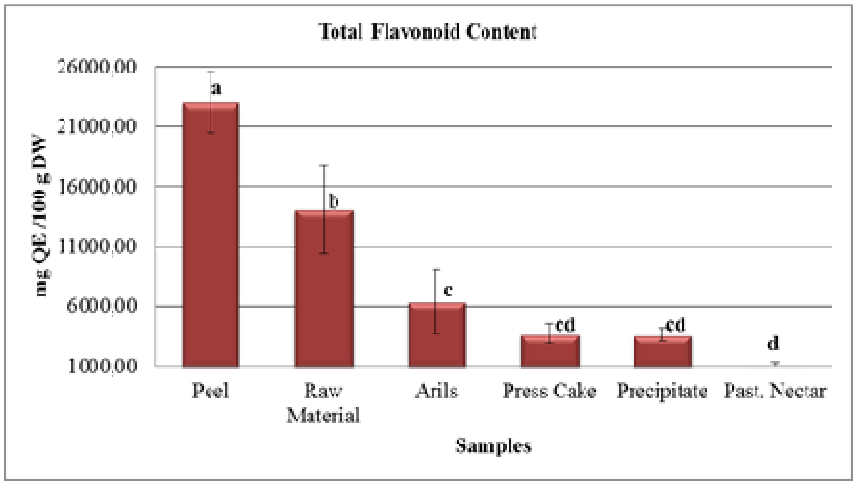


Figure 4.6: Total flavonoid content of raw materials, waste products and the product.

Martos et al. (2011) reported total phenolic content of pomegranate juice arils bagasse and whole fruit bagasse (5.71 ± 0.29 and 7.19 ± 0.46 mg rutin equivalents/g sample, respectively). In this study total flavonoid content of press cake was calculated as 18.02 ± 2.84 mg QE/g sample which was higher than their study and distinct can be due to difference in standards. Ardekani et al. (2011) reported that total flavonoid content for the peel extract in the range of 18.61 ± 0.53 to 36.40 ± 1.34 mg CE/g dry extract and Li et al. (2006) found 59.1 ± 4.8 mg rutin /g fresh extract. In this study, total flavonoid content of peel was found as 230.05 ± 25.42 mg QE/g DW and 70.83 ± 8.20 mg QE/g FW which was closer to Li et al., but higher than Ardekani et al. because of the difference in standards. Karadeniz et al. (2005) found total flavonoid content of whole pomegranate as 45.9 ± 6.7 mg CE/100 g FW, however, in this study it was found as 3801 ± 1388.0 mg CE/100 g FW. Although evaluation is with the same standard, supplying pomegranate from processing plant instead of markets caused the important difference. Because it is known that storage conditions and time can cause loss of phenolics and flavonoids (Yuksel and Koca, 2008).

Khandere et al. (2011) reported that enzyme (pectinase) application before pressing in black carrot increased the flavonoid content by 46%. After a decrease in pasteurization, pectinase application also increased the flavonoid content significantly in this study.

4.3 Total Anthocyanin Content

Total anthocyanin content results were expressed as mg cyanidin-3-glucoside (cyn-3-gly)/100 g DW. Results and statistical evaluation of total anthocyanin content for all samples are shown in Table 4.3.

Table 4.3: Total anthocyanin contents for all samples.

Sample	mg cyn-3-gly/100 g DW
PP	52.60 ± 11.77 e
RM	144.70 ± 28.10 abc
PA	176.60 ± 24.96 a
PM	130.70 ± 53.25 bcd
PR	151.10 ± 16.57 abc
PC	48.40 ± 9.04 e
CO	162.00 ± 21.47 ab
PAS	129.90 ± 2.25 bcd
EA	149.40 ± 18.77 abc
CL	119.80 ± 27.68 cd
PAC	126.70 ± 3.14 bcd
UF	100.50 ± 14.20 d
CON	101.00 ± 10.86 d
NE	34.70 ± 7.07 e
PN	26.30 ± 3.30 e

Arils showed the highest total anthocyanin content value and pasteurized nectar the lowest, however, there was no significant difference between peel, press cake, nectar and pasteurized nectar ($p>0.05$). Pomegranate peel does not have high values for total anthocyanin although it has highest values for total phenolic and total flavonoid. According to evaluation of all samples, enzyme application, cooling, pressing, raw material and arils showed the same anthocyanin content. After arils and raw material, cooling showed the highest value due to protection of anthocyanins at low temperatures which was not so much significantly different when compared to them.

Change in total anthocyanin content during production of pasteurized nectar is shown in Figure 4.7. There was an important decrease from evaporation to nectar production. Total anthocyanin content did not change for raw material, mashing, pressing, cooling, pasteurization, enzyme application and evaporation, significantly ($p>0.05$).

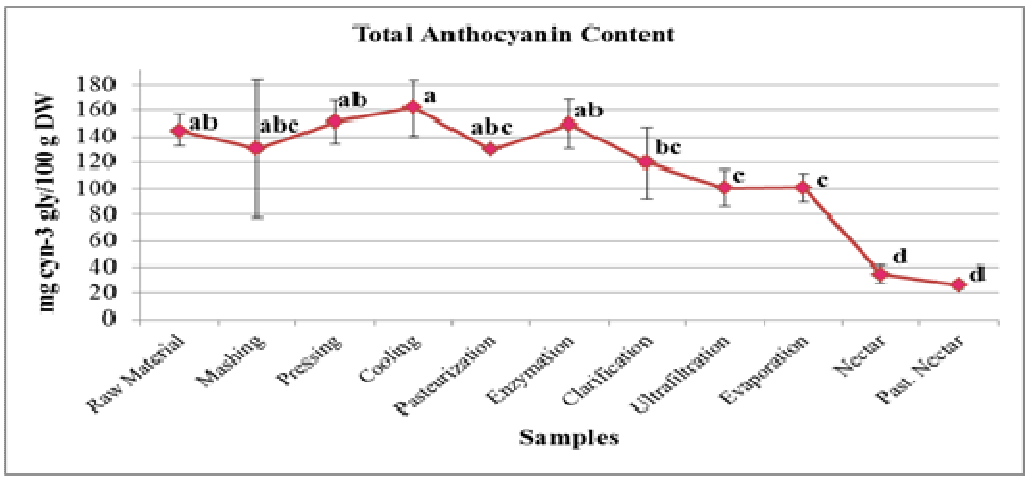


Figure 4.7: Change in total anthocyanin content during pomegranate nectar production.

When raw material, the product, waste products and arils were evaluated together as shown in Figure 4.8, it was obvious that arils had the highest and pasteurized nectar the lowest values. Total anthocyanin contents of both raw material and the precipitate; and peel and the press cake were similar statistically in between.

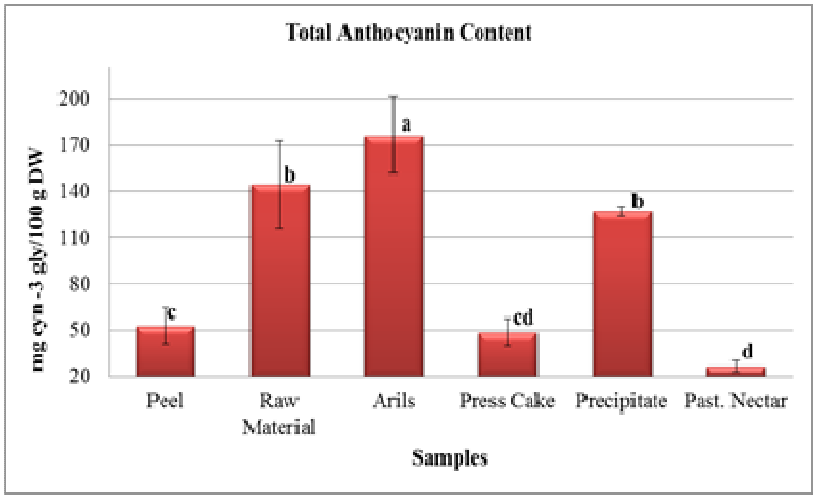


Figure 4.8: Total anthocyanin content of raw materials, waste products and the product.

Nasr et al. (1996) evaluated the effect of shade and sun on the anthocyanin content of peel and they reported their values as 52.00 ± 2.00 and 103.00 ± 3.00 mg/100g DW, respectively. In this study, pomegranate peel showed total anthocyanin content as 52.60 ± 11.77 mg cyn-3-gly/100 g DW which was consistent with the value in shade. Elfalleh et al. (2011) reported total anthocyanin content of Tunisian pomegranate peel as 73.90 ± 8.24 mg cyn-3-gly/g DW which was higher than the value found in this study. Pomegranate variety and growth conditions are effective factors for

phenolic content (Fazzari et al., 2008). Moreover, the results about total anthocyanin content of pomegranate in literature may be different due to evaluation according to dry or fresh weight.

Klopotek et al. (2005) studied changes in total anthocyanins by pH differential method during processing strawberries to different product such as juice, nectar, wine and puree and they found different result from this study as pasteurization and mashing were the effective methods that caused significant reductions. Although reductions in the same processes were observed in this study for pomegranate, statistical evaluation showed that it was not significant.

Turfan et al. (2008) investigated the effects of clarification, pasteurization and concentration on anthocyanin content in laboratory conditions and found 19%, 9% and 8% reductions, respectively. Using gelatin in clarification caused a decrease, significantly. In this study clarification did not cause a significant reduction like that maybe because of industrial scale production.

4.4 Total Tannin Content

Standard calibration curve of catechin seen in Figure 4.9 was prepared. Results of total tannin content analysis were expressed as mg catechin equivalents (CE)/100 g DW for each sample. The standard calibration curve was prepared between 0.005-0.06 mg/ml concentration and the equation was used to calculate the absorbance values of the samples measured by spectrophotometer.

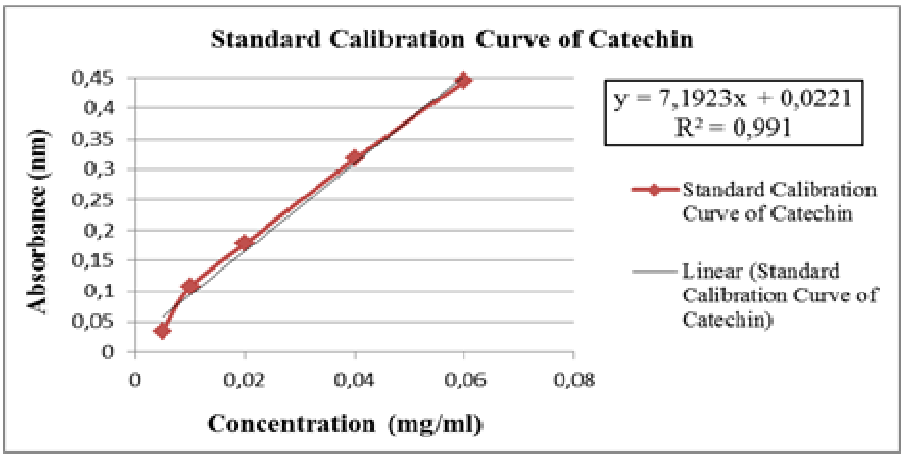


Figure 4.9: Standard calibration curve of catechin.

Total tannin content of all samples was evaluated statistically as shown in Table 4.4. According to this table, raw material showed the highest value, however, there was no significant difference between peel and raw material. Pasteurized nectar had the lowest value and it was not different from nectar, concentrate and pressing. Arils, mashing, press cake, pasteurization, enzyme application, precipitate after clarification and ultrafiltration showed no difference, statistically.

Table 4.4: Total tannin contents for all samples.

Sample	mg CE/100 g DW
PP	1563.40 ± 365.36 a
RM	1883.10 ± 617.13 a
PA	853.60 ± 225.53 b
PM	747.00 ± 171.13 bc
PR	380.60 ± 46.28 cd
PC	498.80 ± 261.02 bc
CO	451.60 ± 88.75 cd
PAS	737.70 ± 60.04 bc
EA	593.70 ± 59.20 bc
CL	393.4 ± 245.09 cd
PAC	518.9 ± 140.65 bc
UF	561.00 ± 100.13 bc
CON	438.50 ± 99.14 cd
NE	106.60 ± 22.91 d
PN	86.60 ± 13.70 d

Change in total tannin content during production was evaluated as shown in Figure 4.10. Discarding peel which is a good source for tannin caused an important decrease from raw material to mashing. On the other hand production from mashing to evaporation did not cause a significant change. Furthermore, besides having the lowest value, pasteurized nectar had similar tannin contents with nectar, pressing, cooling, clarification and evaporation samples.

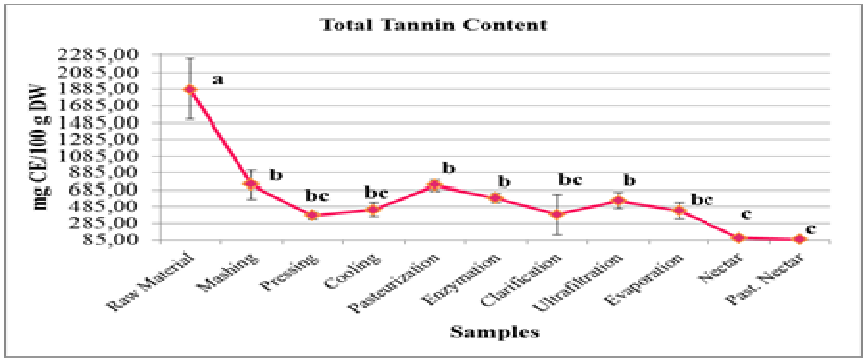


Figure 4.10: Change in total tannin content during pomegranate nectar production.

As reported in Figure 4.11 when raw material, arils, product and waste products were evaluated together, no difference was found between peel and raw material. However they both were all significantly different from others. Arils, press cake and precipitate were found to be similar and pasteurized nectar was not different from press cake and precipitate although it was different from arils.

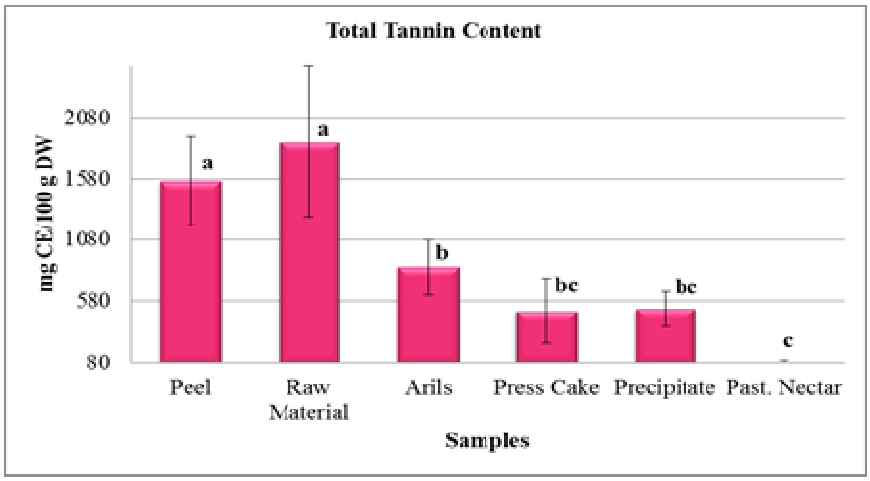


Figure 4.11: Total tannin content of raw materials, waste products and the product.

Martos et al. (2011) reported total tannin contents of pomegranate juice bagasse and pomegranate juice whole fruit bagasse as 669.00 ± 33.00 and 818.00 ± 14.00 mg CE/100 g sample. In this study, press cake showed total tannin content as 238.70 ± 112.90 mg CE/100 g sample which was different from Martos et al., due to difference in pomegranate variety and production procedure.

Mousavinejad et al. (2009) quantified total tannin content of pomegranate juices produced in laboratory conditions as 0.015-0.030 g /100 g sample, which was lower than this study maybe because of using different raw material, analyzing with different methods and producing with different techniques.

4.5 Total Antioxidant Activity

4.5.1 Total antioxidant activity by CUPRAC method

Trolox standard calibration curve was prepared for CUPRAC method as shown in Figure 4.12 and the results were expressed as mg Trolox equivalents (TEAC)/100 g DW for each sample. The standard calibration curve was prepared between 0.07-0.9 mg/ml and the equation was used to calculate the absorbance values of the samples measured by spectrophotometer.

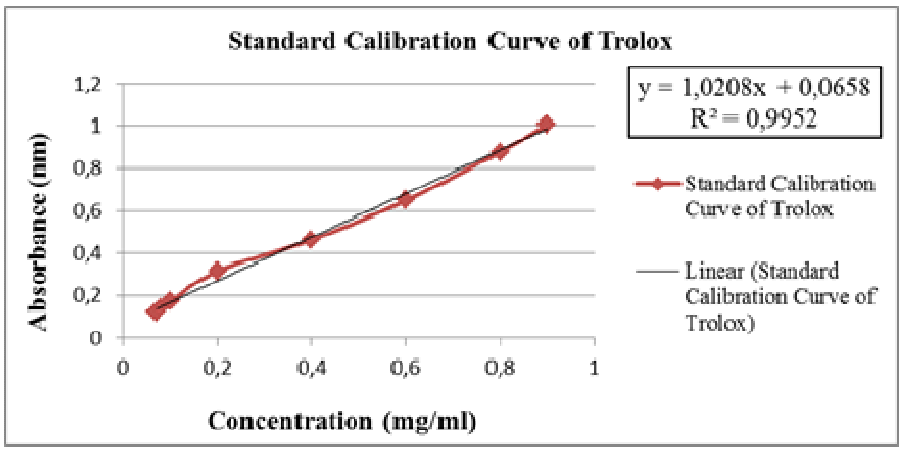


Figure 4.12: Standard calibration curve of Trolox for CUPRAC method.

Antioxidant activity results were calculated for all samples as shown in Table 4.5. Peel showed the highest value as 90876.26 ± 10301.88 and pasteurized nectar showed the lowest as 4313.41 ± 879.92 mg TEAC/100 g DW. The greatest reductions were from raw material to mashing and from evaporation to nectar. There was no significant difference between arils and all process steps from mashing to evaporation ($p>0.05$).

Table 4.5: Total antioxidant activity analysis by CUPRAC for each steps.

Sample	mg TEAC/100 g DW
PP	90876.26 ± 10301.88 a
RM	60677.08 ± 20300.90 b
PA	23472.80 ± 9207.14 c
PM	19899.66 ± 6220.87 cd
PR	9385.75 ± 639.90 de
PC	14167.94 ± 3129.42 cde
CO	11677.13 ± 2603.48 cde
PAS	17808.90 ± 1645.52 cd
EA	15090.78 ± 3757.20 cde
CL	13606.61 ± 5571.66 cde
PAC	16133.05 ± 2611.86 cde
UF	9286.83 ± 4418.84 de
CON	16613.28 ± 1352.57 cde
NE	4550.34 ± 1388.23 f
PN	4313.41 ± 879.92 f

Change in total antioxidant activity by CUPRAC method during production is shown in Figure 4.13. After rapid decrease from raw material to mashing, total antioxidant activity did not change during production, significantly. Nectar and pasteurized nectar showed similar values when they were compared with pressing, cooling, enzyme application, clarification, ultrafiltration and evaporation.

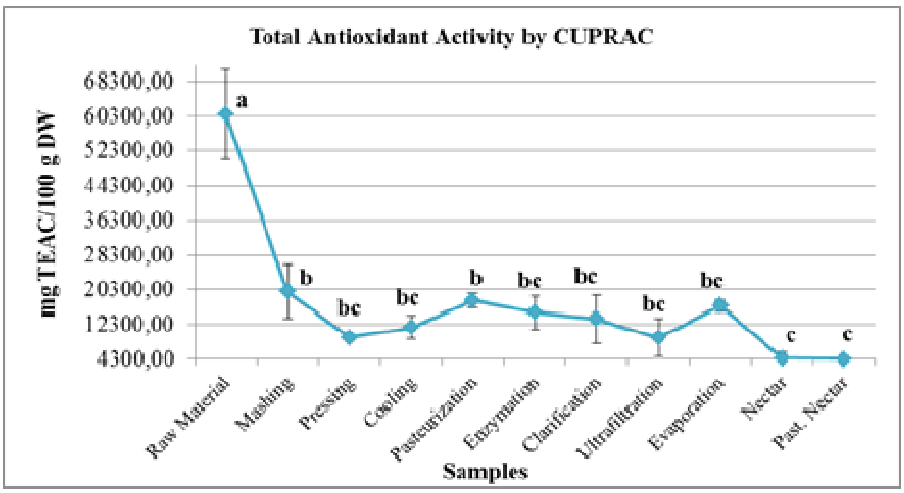


Figure 4.13: Change in CUPRAC during pomegranate nectar production.

When raw material, arils and pasteurized nectar were compared with waste products as presented in Figure 4.14, it was observed that press cake and precipitate had total antioxidant capacity as high as arils but not significantly lower than the peel and the raw material. Moreover, pasteurized nectar showed the same antioxidant activity as press cake and precipitate. Significant levels of antioxidant activity were lost by discarding peels.

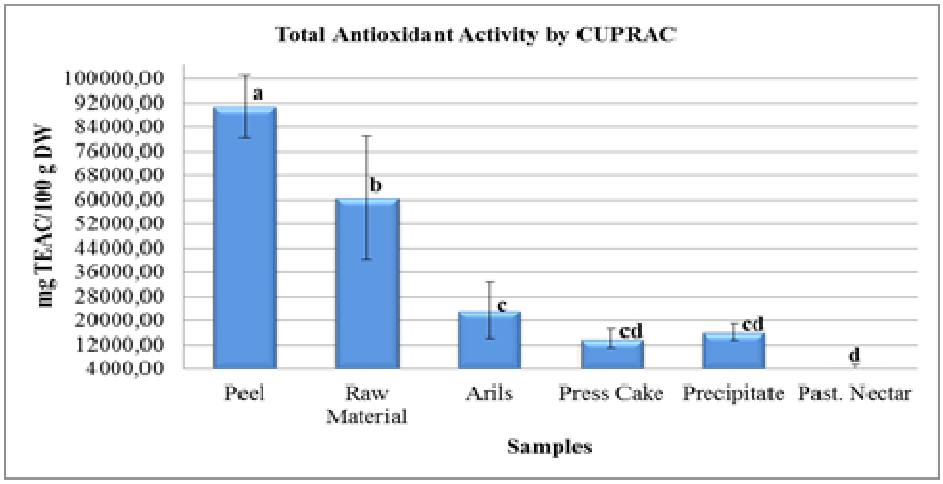


Figure 4.14: Total antioxidant activity by CUPRAC for raw materials, waste products and the final product.

CUPRAC is a new method and its application to different foods has been proceeding. Although it has not been used in pomegranate juice, there are some studies focusing on other fruits. Khandare et al. (2011) used CUPRAC method to study the effect of processing on antioxidant composition during black carrot juice production. The changes were found to be dependent on the enzyme (pectinase) concentration. Antioxidant activity increased 30% when it was 0.25 ml/kg, but at higher

concentrations, there was a significant decrease in antioxidant activity. However; enzyme application did not create significant changes from mashing to evaporation during pasteurized pomegranate juice production.

Capanoglu (2008b) studied on changes in antioxidant profiles during production of tomato paste and according to CUPRAC results, it was claimed that seed and skin showed higher value than fruit and during production of tomato paste antioxidant activity decreased. However, production of pasteurized pomegranate juice did not increase total antioxidant capacity, except from raw material to mashing.

4.5.2 Total antioxidant activity by DPPH method

Standard calibration curve of Trolox was prepared for DPPH method as shown in Figure 4.15. The results were expressed as mg TEAC/100 g DW for each sample. The standard calibration curve was obtained between 0.008-0.2 mg/ml and the equation was used to evaluate the absorbance values of the samples measured by spectrophotometer.

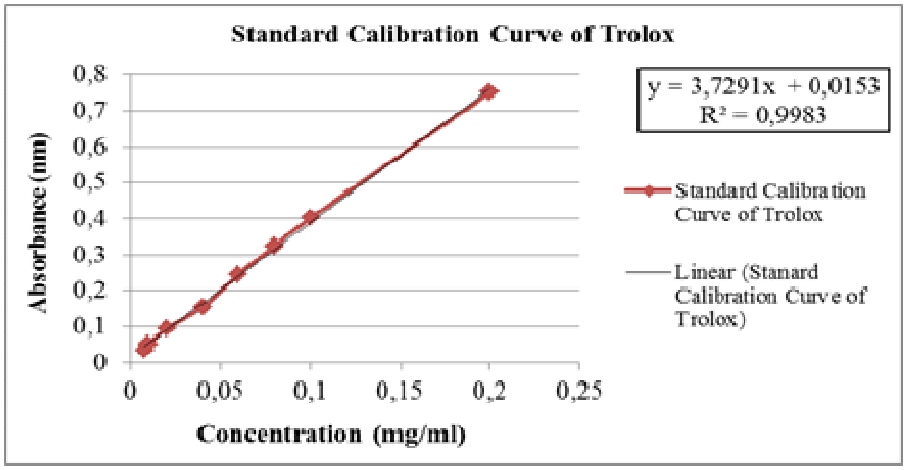


Figure 4.15: Standard calibration curve of Trolox for DPPH method.

All the samples were evaluated according to their total antioxidant activity analysis results by DPPH. Pomegranate peel showed the highest and pasteurized pomegranate nectar had the lowest as 42884.98 ± 5798.35 and 1843.497 ± 246.62 mg TEAC/100 g DW, respectively as presented in Table 4.6. Arils, mashing, cooling, enzyme application and clarification showed the same values. Change in total antioxidant activity by DPPH method during production is shown in Figure 4.16.

Table 4.6: Total antioxidant activity analysis by DPPH for each steps.

Sample	mg TEAC/100 g DW
PP	42884.98 ± 5798.35 a
RM	26326.97 ± 6725.78 b
PA	11829.58 ± 3087.96 c
PM	10177.56 ± 1818.32 cd
PR	7164.121 ± 1197.35 de
PC	6147.401 ± 591.75 def
CO	8998.372 ± 1352.69 cde
PAS	5201.4 ± 175.25 ef
EA	8910.06 ± 1691.94 cde
CL	8431.313 ± 1778.81 cde
PAC	6865.506 ± 875.86 de
UF	7002.113 ± 1850.21 de
CON	7040.323 ± 945.35 de
NE	2113.143 ± 492.72 f
PN	1843.497 ± 246.62 f

The trend was similar to the one obtained in CUPRAC method but the only difference was that production of nectar from concentrate caused a significant decrease and total antioxidant activity of pasteurization step was as low as in nectar and pasteurized nectar. There was generally no significant change during production from mashing to evaporation as obtained by CUPRAC method.

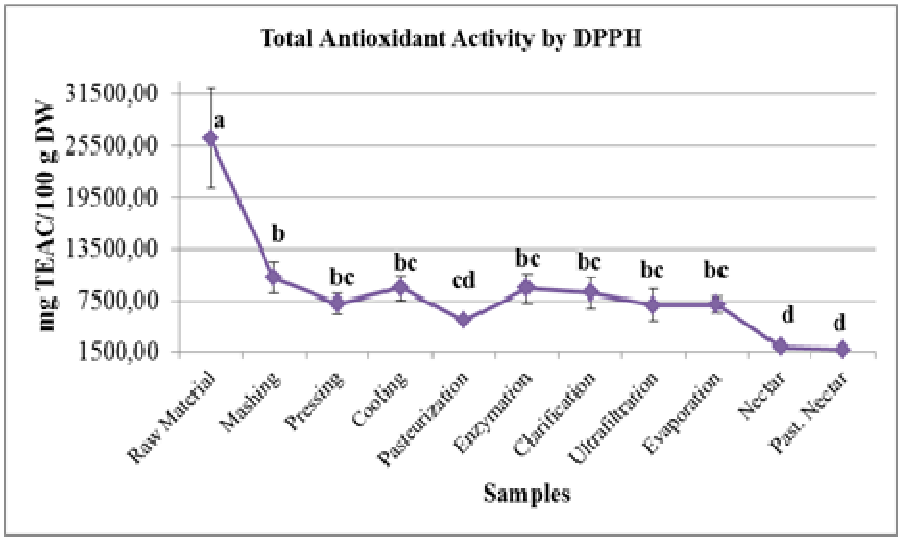


Figure 4.16: Change in DPPH during pomegranate nectar production.

As shown in Figure 4.17, total antioxidant activity by DPPH method was not different for arils, press cake and precipitate. Pasteurized nectar was found to be similar to press cake and precipitate. Peel and raw material were higher than others as expected. Significant levels of antioxidant activity were lost by discarding peels.

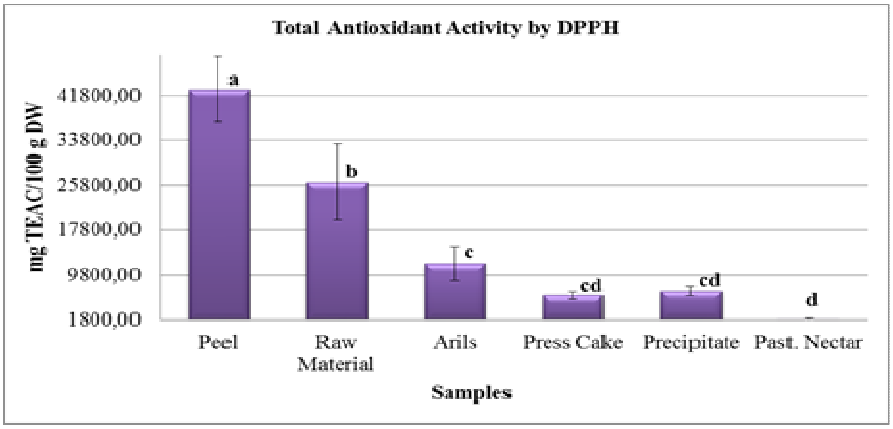


Figure 4.17: Total antioxidant activity by DPPH for raw materials, waste products and the final product.

Izquierdo et al. (2002) used DPPH method to measure the effects of pasteurization, concentration and freezing on antioxidant capacity during industrial scale orange juice production and they reported that pasteurization, concentration and freezing did not cause significant differences.

Capanoglu et al. (2008) also used DPPH method to observe changes in antioxidant profiles during production of tomato taste. Production of tomato paste did not change total antioxidant acitivity by DPPH method, however seed and skin had lower values than fruit, differently from CUPRAC.

4.5.3 Total antioxidant activity by FRAP method

Trolox standard calibration curve was prepared for FRAP method as shown in Figure 4.18 and the results were expressed as mg TEAC/100g DW. The standard calibration curve was prepared between 0.01-0.1 mg/ml and the equation was used to determine the absorbance values of the samples measured by spectrophotometer.

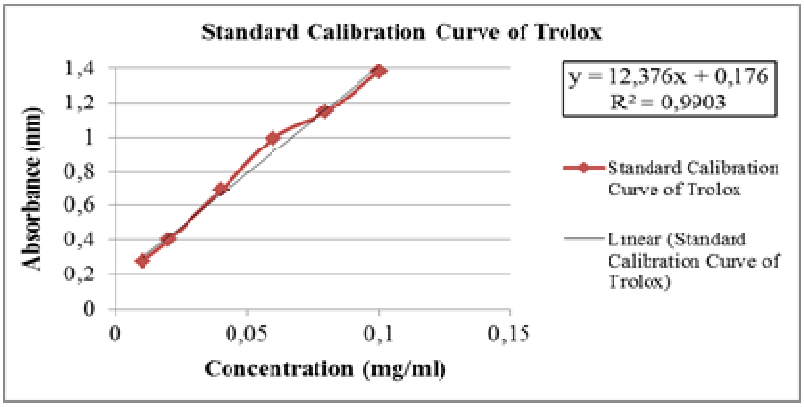


Figure 4.18: Standard calibration curve of Trolox for FRAP Method.

Peel showed the highest and pasteurized nectar the lowest values as 26622.14 ± 3473.13 and 1650.17 ± 304.58 mg TEAC/100 g DW, respectively. Press cake, pasteurization, nectar and pasteurized nectar had similar values.

Results of total antioxidant activity analysis by FRAP method for all samples are shown in Table 4.7. Arils showed the same level of antioxidant activity as other processing steps, except for nectar production and pasteurized nectar.

Table 4.7: Total antioxidant activity analysis by FRAP for each steps.

Sample	mg TEAC/100 g DW
PP	26622.14 ± 3473.13 a
RM	15754.89 ± 4255.89 b
PA	7234.32 ± 2231.04 c
PM	6895.90 ± 764.12 c
PR	5990.26 ± 1150.06 c
PC	4225.61 ± 1070.58 cd
CO	7192.88 ± 825.80 c
PAS	4112.80 ± 2052.58 cd
EA	7337.24 ± 654.66 c
CL	6732.62 ± 2418.38 c
PAC	5882.41 ± 966.53 c
UF	5938.77 ± 1076.09 c
CON	6425.46 ± 1523.76 c
NE	1861.03 ± 277.05 d
PN	1650.17 ± 304.58 d

Change in total antioxidant activity by FRAP method during production is shown in Figure 4.19. There were significant reductions from raw material to mashing, from cooling to pasteurization and from evaporation to nectar. Pasteurization showed lowest values as nectar and pasteurized nectar, differently from CUPRAC and DPPH. There was no change from mashing to evaporation, except for pasteurization.

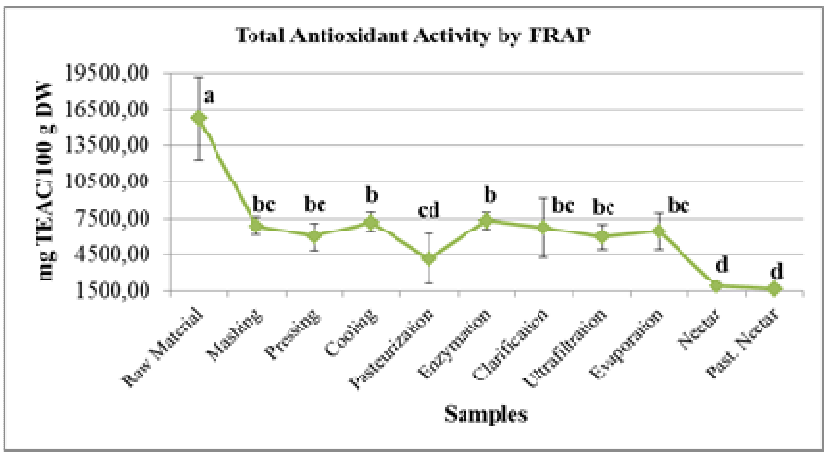


Figure 4.19: Change in FRAP during pomegranate nectar production

According to evaluation of peel, raw material, pasteurized nectar and waste products as presented in Figure 4.20, there were similar changes with the ones obtained by CUPRAC and DPPH methods. Arils, press cake and precipitate showed the same antioxidant levels ($p>0.05$) and peel showed significantly highest antioxidant activity than arils and raw material ($p<0.05$).

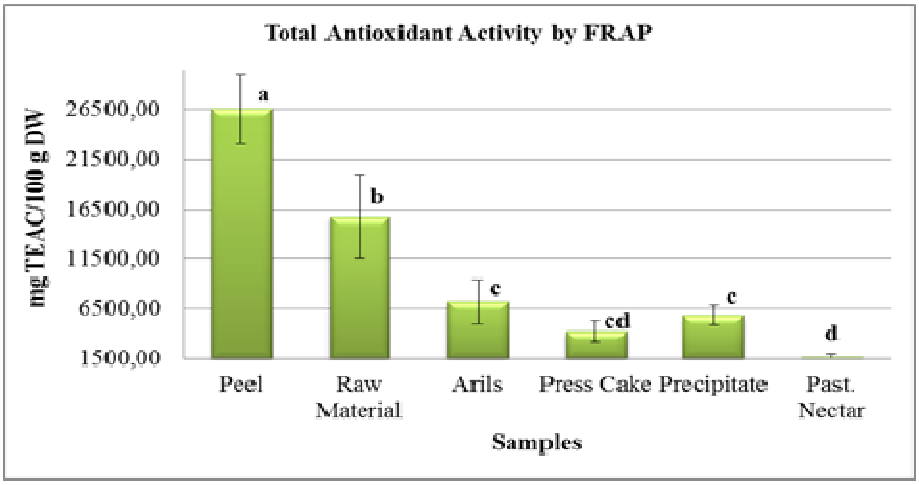


Figure 4.20: Total antioxidant activity by FRAP for raw materials, waste products and the final product.

Elfalleh et al. (2011) reported FRAP value of peel as 25.63 ± 3.67 mmol/100g DW whereas Ardekani et al. (2011) determined between 27.93 ± 7.68 and 46.7.817 ± 10.81 mmol/100g DW. Wootton-Beard & Lisa Ryan (2011) found FRAP value of whole pomegranate as 19400 µmol/kg. Due to difference in pomegranate fruit variety and maturity, phenolic compounds composition and antioxidant acitivity can be affected.

According to evaluation of changes of FRAP values during production of tomato paste by Capanoglu et al. (2008), seed and skin showed lower value and production of paste did not cause an important change as observed for pomegranate in this study.

4.5.4 Total antioxidant activity by ABTS method

Standard calibration curve for ABTS was prepared by using Trolox as as shown in Figure 4.21 and the results were expressed as mg TEAC/100 g DW. The calibration curve was obtained between 0.01-0.1 mg/ml and the equation was used to evaluate the absorbance values of the samples.

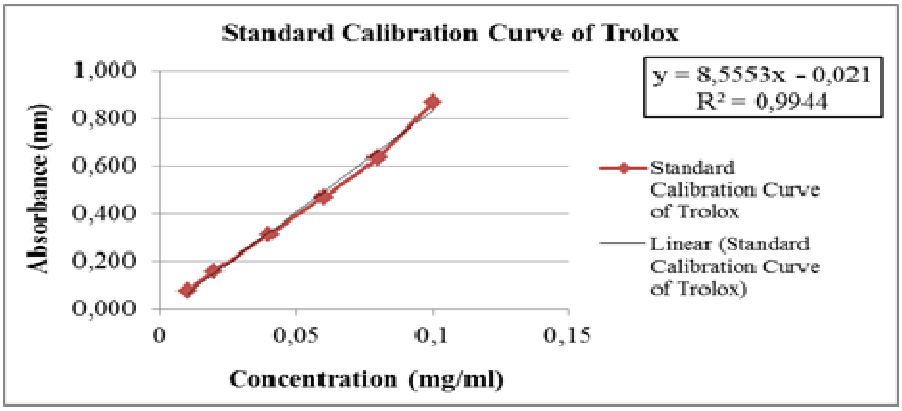


Figure 4.21: Standard calibration curve of Trolox for ABTS method.

Results of ABTS analysis for all samples were calculated as presented in Table 4.8.

Table 4.8: Total antioxidant activity analysis by ABTS for each steps.

Sample	mg TEAC/100 g DW
PP	51100.85 ± 2404.38 a
RM	30762.61 ± 5245.41 b
PA	14942.99 ± 3542.30 c
PM	9481.606 ± 1013.46 d
PR	10107.95 ± 1134.07 d
PC	3939.958 ± 70.98 ef
CO	11169.97 ± 799.56 d
PAS	2563.2 ± 96.21 ef
EA	10679.74 ± 1521.75 d
CL	9195.674 ± 1304.12 d
PAC	5132.205 ± 366.80 e
UF	8531.107 ± 1823.01 d
CON	10933.33 ± 1444.58 d
NE	1421.158 ± 274.04 f
PN	1177.363 ± 239.17 f

Peel showed the highest value as 51100.85 ± 2404.38 and pasteurized nectar had the lowest as 1177.363 ± 239.17 mg TEAC/100 g DW, as observed in other total antioxidant activity methods. On the other hand, samples taken from press cake and pasteurization steps did not differ significantly from nectar and pasteurized nectar. Arils had significantly higher antioxidant activity than processing steps ($p<0.05$).

Change in total antioxidant activity by ABTS method during production is shown in Figure 4.22. After raw material to mashing, there was no significant change during production from mashing to evaporation, except for pasteurization, and this was a different result when compared to other methods. Pasteurization and nectar

production significantly reduced antioxidant activity ($p<0.05$). No important difference was observed during pasteurization of nectar.

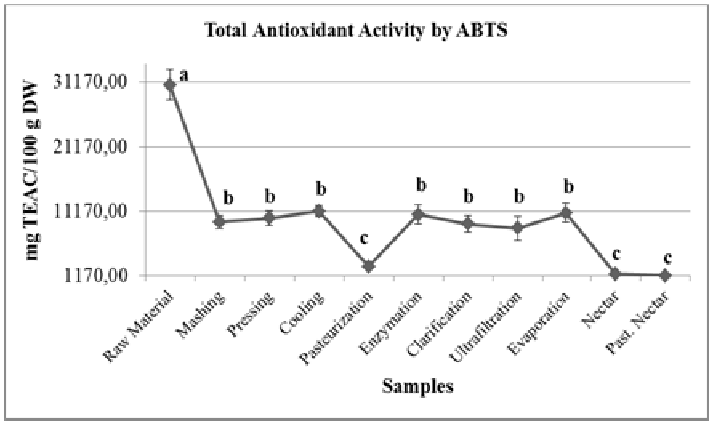


Figure 4.22: Change in ABTS during pomegranate nectar production.

When Capanoglu et al. (2008) researched the effect of processing of tomato to its paste using antioxidant activity by ABTS method, they reported that no important changes were observed during production including pasteurization and there was no change between fruit and seed and skin. On the other hand, Klopotek et al. (2005) reported the same argument consistent with this study as antioxidant activity by ABTS method was negatively affected in pasteurization and mashing steps, especially, during processing strawberries to different products such as juice, nectar, wine and puree.

As presented in Figure 4.23, total antioxidant activity by ABTS of raw material, peel, arils, waste products and the product were evaluated. Press cake, precipitate and pasteurized nectar was not different from each other. Peel had higher total antioxidant activity than raw material and arils.

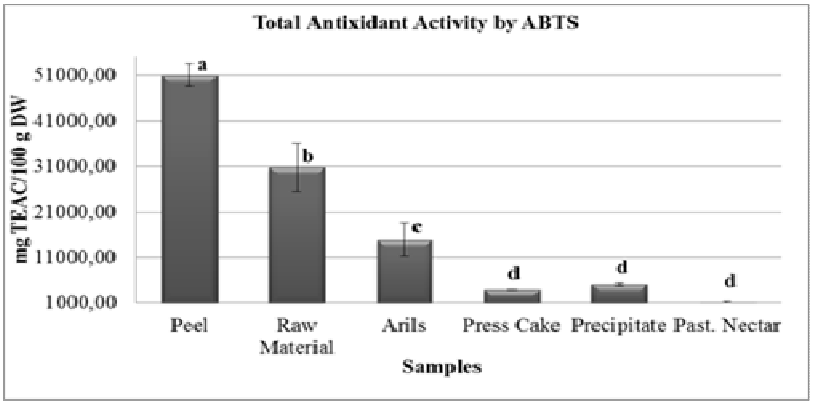


Figure 4.23: Total antioxidant activity by ABTS for raw materials, waste products and the final product.

For all antioxidant activity methods, peel and whole material showed higher values. Commonly found anthocyanins are cyanidin glucosides which have high antioxidant capacity due to their o-dihydroxy structure and the reason for higher activity of pomegranate is explained by this compound (Karadeniz et al., 2005).

4.6 Polymeric Color (%) Analysis

Polymeric color (%) results of each sample, except nectar, were calculated as shown in Table 4.9.

Table 4.9: Results of polymeric color (%) analysis for all samples.

Sample	Polymeric Color (%)
PP	94.23 ± 4.43 ab
RM	85.64 ± 11.73 ab
PA	79.88 ± 23.36 abc
PM	79.26 ± 21.76 abc
PR	86.37 ± 3.4 ab
PC	91.27 ± 7.03 ab
CO	62.59 ± 19.81 c
PAS	98.75 ± 5.11 a
EA	96.14 ± 5.49 ab
CL	85.34± 10.2 ab
PAC	93.13 ± 6.68 ab
UF	77.06 ± 14.84 abc
CON	97.73 ± 8.8 ab
PN	76.03 ± 11.56 bc

The results expressed as % mean value ± SD and the differences between the samples were determined statistically.

Arils, mashing, ultrafilration, cooling and pasteurized nectar showed similar values. Pasteurization showed higher polymeric color (%) than peel and raw material but there was no significant difference ($p>0.05$).

Changes in polymeric color during production are shown in Figure 4.24. Generally, there was no significant change, except for the cooling step. However, cooling showed similar values with pasteurized nectar. Polymeric color values did not excess 100% but reached maximum level at pasteurization and minimum at cooling.

The increase in polymeric colour after pasteurization may be due to the polymerization and degradation of anthocyanins during heating (Gil et al., 2000).

There is an increase for polymeric color during processing, especially, heat treatment as reported in literature. Polymeric color values increased during processing berry fruits to products due to reaction between anthocyanin and tannin to from polymeric pigments (Howard et al., 2012).

Pomegranate, which showed the highest value for total tannin content analysis is a rich source for tannin, so the reaction between anthocyanin and tannin cause an increase in polymeric color (%).

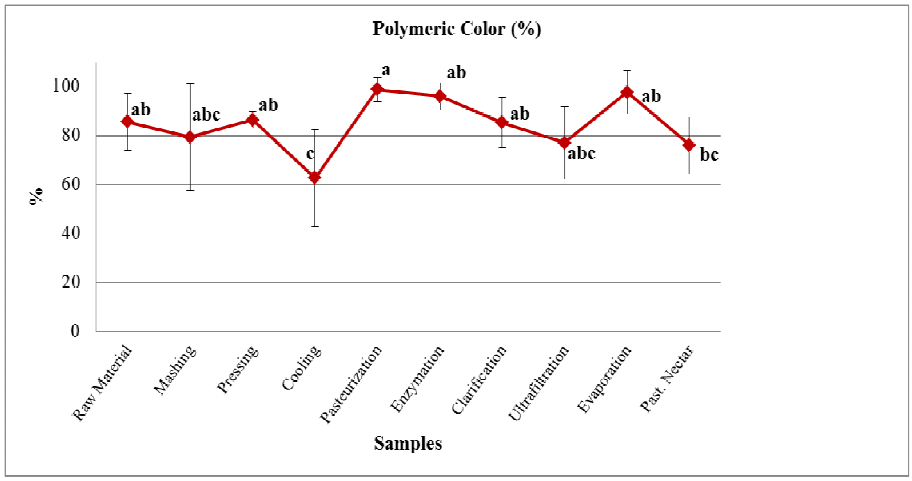


Figure 4.24: Changes in polymeric color during pomegranate nectar production.

When polymeric color (%) was evaluated according to raw material, arils, press cake, precipitate after clarification, peel and pasteurized nectar, it was observed that there was no significant difference between them as presented in Figure 4.25 ($p>0.05$).

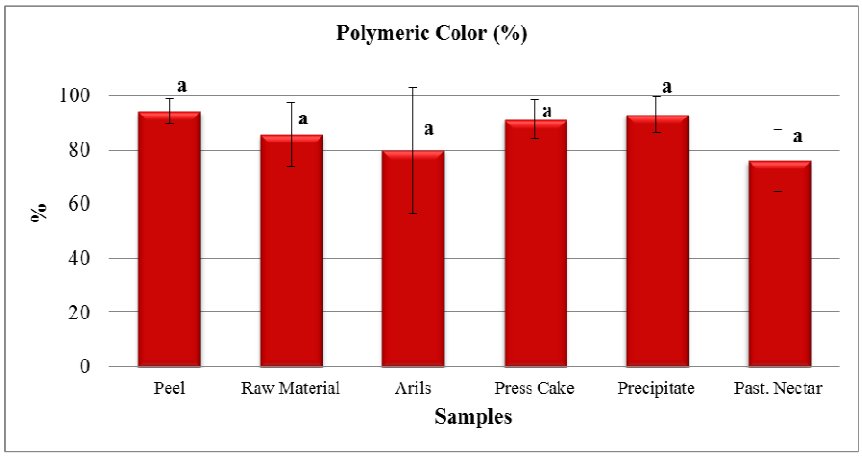


Figure 4.25: Polymeric color of raw materials, waste products and the product.

Alper et al. (2005) researched influence of processing on pomegranate juice and analyzed polymeric color. They claimed that it was high in raw pomegranate juice

(not clarified and not heat treated) but low in UF-treated samples. Pasteurization increased the values by 20.5-35.4%.

Howard et al. (2012) researched processing effect on berry polyphenols. They also analyzed polymeric color and found values between 10.3% (bilberry powder) and 78.4% (concord grape juice). Turfan et al. (2008), studied about the effects of clarification, pasteurization and concentration on pomegranate juice color and anthocyanins. They obtained juices by hand pressing in laboratory conditions. All of the operations increased polymeric color. They explained the increase by formation of brown colored compounds by polymerization of compounds as a result of disintegration of monomeric anthocyanins. They suggested exposing pomegranate juice to heat at minimum level and avoiding utilization of stored pomegranate as the raw material for processing.

Yuksel and Koca (2008) determined polymeric color changes during storage of blackberry nectar at room temperature (20^{o}C) and refrigerator temperature (4^{o}C) for 7 months. The values were higher at 20^{o}C and increased gradually. In this study to prevent any change or loss in phenolic compounds, samples were stored at -80^{o}C.

Turfan et al. (2011) investigated color changes during processing of pomegranate juice from sacs and whole fruit. Polymeric colour was high in unclarified samples (25–29%). Compared to unclarified pasteurized juice, higher polymeric color was formed in clarified pasteurised juice.

Polymeric colour also depends on processing and storage conditions and may be higher than 30% if fruits or vegetables are exposed to undesirable storage conditions (Turfan et al., 2011).

4.7 Results of Major Phenolic Compounds Analysis by HPLC-PDA

Phenolic and anthocyanin profiles of each extract were determined by using RP-HPLC/PDA. Retention times were determined and calibration curves of standards given in Figure B.1-B11 were used to form equations and obtain the factors. HPLC-DAD chromatograms for each sample were extracted at 280, 312, 360 and 520 nm wavelengths. Representative HPLC-DAD (monitored by selected wavelength) chromatograms of each sample were presented in Figure C1-C60.

As a result of the evaluation of HPLC chromatograms, gallic acid, ferulic acid, q-3-BDg, del 3,5-dOg and cyn-3-O-glu were identified for all samples including processing steps, products and waste products. However, the other anthocyanins and phenolic acids could not be found for all samples.

4.7.1 Results of major phenolic acid analysis by HPLC-PDA

Changes in catechin, q-3-g, q-3-BDg, gallic acid and ferulic acid are showed in Table 4.10.

Table 4.10: Evaluation of major phenolic compound concentration of all samples.

Phenolic	Gallic Acid	Ferulic Acid	Q-3-BDG	Q-3-G	Catechin
Sample	mg/100g DW	mg/100g DW	mg/100g DW	mg/100g DW	mg/100g DW
PP	417.67 ± 355.43 a	54.94 ± 18.17 b	447.07 ± 311.11 a	117.15 ± 12.41 a	8101.31 ± 2078.26 a
RM	36.09 ± 5.35 b	74.06 ± 15.58 a	50.57 ± 26.71 de	68.53 ± 40.61 b	6400.22 ± 2059.23 a
PA	142.59 ± 28.93 b	47.56 ± 4.23 bc	264.15 ± 74.40 bc	9.14 c	506.48 ± 96.47 b
PM	97.88 ± 10.88 b	39.38 ± 8.53 cd	287.33± 38.73 b	34.6 bc	339.17 b
PR	135.90 ± 29.65 b	30.13 ± 6.67 def	195.04 ± 59.63 bcde	ND	ND
PC	54.63 ± 19.11 b	24.88 ± 4.98 def	105.86 ± 21.60 cde	15.28 ± 3.33 c	289.55 ± 89.77 b
CL	164.11 ± 19.23 b	33.42 ± 7.35 de	210.68 ± 26.63 bcd	6.82 c	40.07 ± 2.28 b
PAS	115.52 ± 3.01 b	40.07 ± 21.81 def	174.39 ± 38.38 bcde	4.98 c	ND
EA	98.12 ± 20.57 b	32.66 ± 6.14 de	152.40 ± 57.47 cde	7.31 ± 0.50 c	ND
CL	85.95 ± 31.96 b	24.35 ± 7.65 ef	103.76 ± 56.77 cde	4.88 ± 0.11 c	ND
PAC	65.91 ± 9.34 b	20.56 ± 1.92 efg	98.27 ± 21.55 cde	ND	ND
UF	78.31 ± 19.56 b	17.64 ± 3.01 fgh	25.46 ± 9.68 e	ND	110.09 b
CON	100.41 ± 10.43 b	28.84 ± 8.29 def	67.41 ± 26.80 de	ND	ND
NE	31.03 ± 8.65 b	7.38 ± 2.78 gh	30.53 ± 11.23 e	ND	ND
PN	26.10 ± 2.33 b	5.84 ± 0.58 h	18.26 ± 5.60 e	ND	10.47 b

(ND: Not Determined)

For samples of pressing, pasteurization, enzyme application, clarification, precipitate, concentrate, nectar, catechin could not be detected. Peel and raw material showed similar values and they were significantly higher than others. Pasteurized nectar had the lowest but not significantly different value than arils, mashing, press cake, cooling and ultrafiltration.

Q-3-g concentrations were lower than catechin. Peel showed significantly the highest value. Mashing has the similar value with raw material. There was no difference between arils, mashing, press cake, pasteurization, enzyme application and concentrate.

Peel showed the highest gallic acid value as 417.67 ± 355.43 mg/100g DW and it was the only significantly different sample from others ($p<0.05$). Except for peel, all samples including raw material, arils and pasteurized nectar did not show a significant difference. Raw material had the highest ferulic acid concentration as 74.06 ± 15.58 mg/100g DW. Arils had similar content with peel and mashing. There

was no important difference between pressing, press cake, cooling, pasteurization, enzyme application, clarification and precipitate. Peel showed the highest q-3-BDg concentration as 447.07 ± 311.11 mg/100g DW. From pressing to production of pasteurized nectar, production steps did not change the concentration significantly. Arils had significantly higher concentration than raw material but closer to mashing. Changes in gallic acid, ferulic acid and q-3-BDg concentration during production of pasteurized pomegranate nectar are given in Figure 4.26.

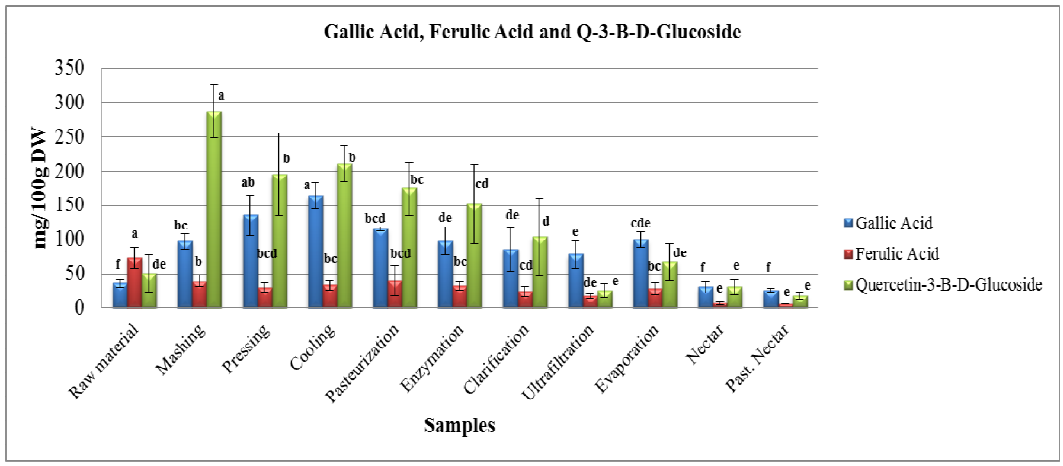


Figure 4.26: Changes in major phenolics content during pasteurized pomegranate nectar producton.

Cooling showed the highest gallic acid concentration, but raw material had low concentration as nectar and pasteurized nectar. There was an increase in gallic acid concentration from raw material to mashing, pressing and cooling.

Bhat et al. reported that hydrolysable tannins caused an increase in gallic acid content (1998). High tannin content of pomegranate increased gallic acid concentration after raw material.

From pasteurization to evaporation, there was no important difference. However, after evaporation it decreased significantly ($p<0.05$). Elfalleh et al. (2011) detected 123.79 ± 9.56 mg/100 g DW gallic acid in Tunusian pomegranate and this value was lower than this study.

There was no change in ferulic concentration from mashing to clarification. Raw material had the highest value. Ultrafiltration was same with nectar and pasteurized nectar. There was an important increase in q-3-BDg concentration from raw material to mashing, however, after mashing it reduced significantly during pressing. It did

not show significant change between pressing and pasteurization. Clarification and ultrafiltration significantly reduced q-3-BDg. Ultrafiltration, evaporation, nectar and pasteurized nectar showed similar values.

Evaluation of q-3-BDg, gallic acid and ferulic acid concentrations of raw material, peel, arils, waste products and the product is shown in Figure 4.27.

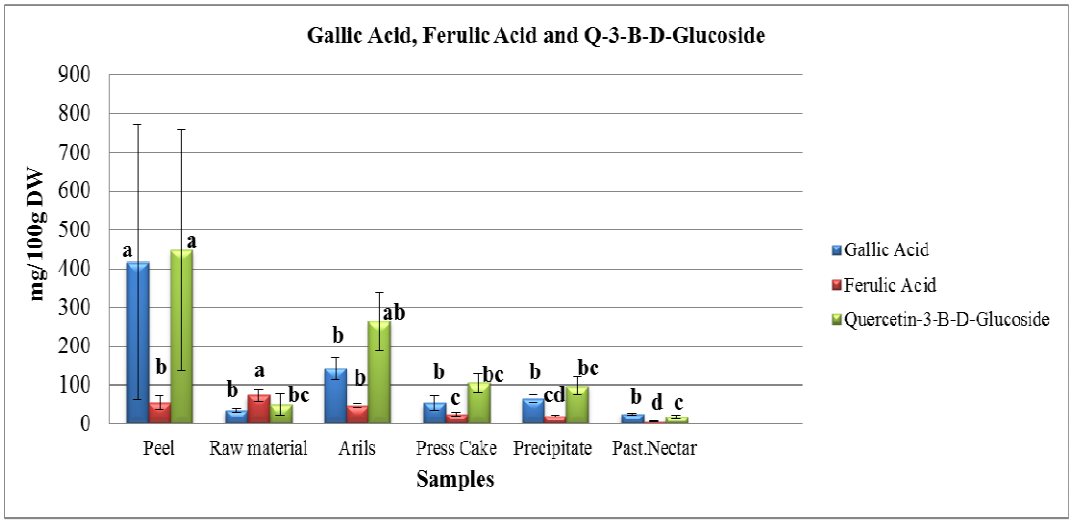


Figure 4.27: Evaluation of gallic acid, ferulic acid, q-3-BDg concentration of raw materials, waste products and product.

No difference was observed for q-3-BDg between peel and arils. On the other hand, raw material, press cake, precipitate and pasteurized nectar showed similar values. According to ferulic acid concentrations, raw material had the highest level, peel and arils were similar statistically and there were no difference between press cake and precipitate. Precipitate was closer to pasteurized nectar. Peel had higher gallic acid concentration than raw material, arils, press cake, precipitate and pasteurized nectar. Although standard deviation of raw material was low, standard deviation of peel was high and it was probably due to difference in peel composition for raw materials of different productions.

Moreoover, p-coumaric acid was detected in peel and cooling step as 23.04, 33.42 ± 7.34 mg/100g DW respectively. Elfalleh et al. (2011) detected p-coumaric acid as 4.48 ± 0.39 mg/100g DW in Tunisian pomegranate which was lower than this research. Neochlorogenic acid was only found in peel for one production as 55.42 mg/100g DW. According to these results, pomegranate peel is also a rich source for other phenolic compounds, but peeling before processing reduces the values during

production. Differences in phenolic acids concentration can be dependent on pomegranate variety and growth conditions.

4.7.2 Results of major anthocyanin analysis by HPLC-PDA

All samples were calculated according to their cyn 3-O-glu, del 3,5-di-O-glu and pel 3,5-di-O-glu concentration as presented in Table 4.11.

Table 4.11: Evaluation of anthocyanin concentrations of all samples.

Anthocyanin	**Kuromanin Chloride (Cyn 3-O-Glu)**	**Delphin Chloride (Del 3,5-dOg)**	**Pelargonin Chloride (Pel 3,5-dOg)**
Sample	**mg/100g DW**	**mg/100g DW**	**mg/100g DW**
PP	11.51 ± 4.34 de	ND	ND
RM	23.26 ± 11.44 cde	31.58 ± 16.97 cde	73.19 ± 38.37 ab
PA	42.63 ± 4.86 ab	37.86 ± 7.19 abcd	103.48 ± 50.44 ab
PM	35.40 ± 9.68 bc	27.73 ± 4.44 defg	69.59 ± 52.05 ab
PR	30.13 ± 6.16 bc	48.96± 12.97 ab	57.02 ± 21.26 ab
PC	20.13 ± 5.58 cde	10.26 ± 1.85 h	17.48 ± 5.83 b
CO	32.83 ± 7.51 bc	53.38 ± 7.74 a	52.28 ± 11.42 b
PAS	52.73 ± 30.08 a	46.07 ± 1.89 abc	149.78 ± 129.08 a
EA	26.47 ± 5.25 bcd	37.08 ± 3.28 abcd	39.62 ± 2.34 b
CL	20.77 ± 7.70 cde	29.87 ± 7.13 cdef	43.37 ± 0.01 b
PAC	22.38 ± 6.91 cde	14.19 ± 8.00 efgh	17.31 ± 5.29 b
UF	18.63 ± 4.40 cde	29.36 ± 9.77 cdef	ND
CON	21.03 ± 0.22 cde	35.56 ± 16.34 bcde	38.28 ± 3.69 b
NE	5.91 ± 2.35 e	14.55 ± 4.76 fgh	ND
PN	5.85 ± 0.84 e	12.09 ± 2.50 gh	ND

(ND: Not Determined)

Cooling had the highest del 3,5-dOg concentration as 53.38 ± 7.74 mg/100g DW. This was probably due to protection of anthocyanins from degradation at low temperatures. It could not be detected in peel; and raw material, arils, pasteurization, enzyme application, clarification, ultrafiltration, concentrate had similar values statistically.

Pasteurization had the highest cyn 3-O-glu and closer concentration to that in arils. Peel showed very low levels and there was no statistical difference between peel, nectar and pasteurized nectar.

Pel 3,5-dOg values were close to each other and pasteurization showed the highest value due to increase in anthocyanin concentration during heat treatment. Arils also had high value, however, it could not be found in peel as expected.

Pel 3-O-glu was only detected in peel and raw material at low amounts as 12.42 ± 0.77 and 3.53 ± 0.92 mg/100g DW, respectively.

Changes in cyn 3-O-glu and del 3,5-dOg concentration during production of pasteurized pomegranate nectar are shown in Figure 4.28.

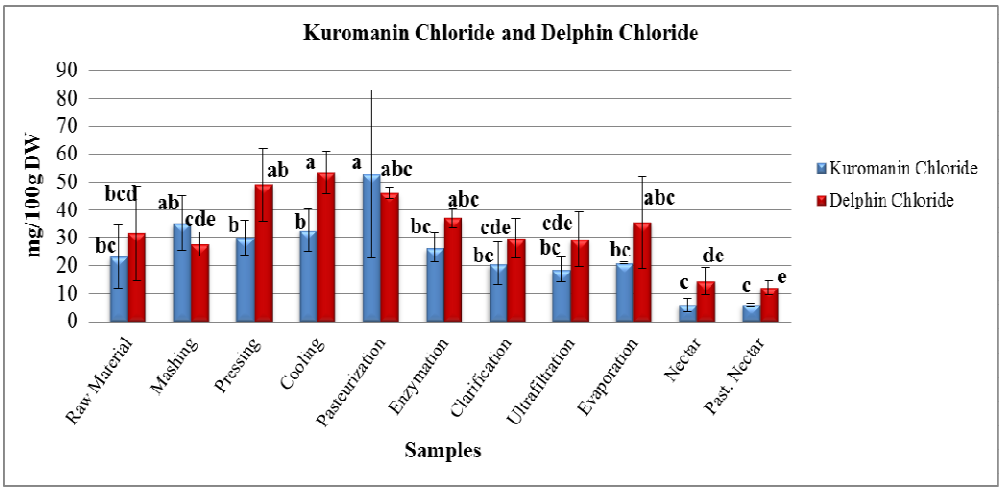


Figure 4.28: Changes in kuromanin chloride and delphin chloride concentration during pomegranate nectar production.

There was no important change for cyn-3-O-glu from raw material to evaporation, except for pasteurization step. Nectar and pasteurized nectar had similar values with raw material, enzyme application, clarification, ultrafiltration and evaporation.

Mashing, clarification, ultrafiltration, nectar and pasteurized nectar showed similar del 3,5-di-O-glu concentration values. No change was observed between pressing, cooling, pasteurization, enzyme application and evaporation. Mashing was significantly different from pressing and cooling. It was clear that pressing and cooling applications significantly increased del 3,5-dOg levels. Holcroft et al. reported that total anthocyanin content of arils increase in cold storage was due to effect of enzyme, phenylalanine ammonia lyase (1998).

Evaluation of cyn 3-O-glu and del 3,5-dOg concentration of raw material, arils, the product and waste products are shown in Figure 4.29. Raw material and arils between each other and press cake, precipitate and pasteurized nectar between themselves had the similar del 3,5-dOg values. Aril showed the highest cyn 3-O-glu concentration and no difference was observed between raw material, press cake and precipitate; and for peel and press cake. Significantly important levels are retained in by products.

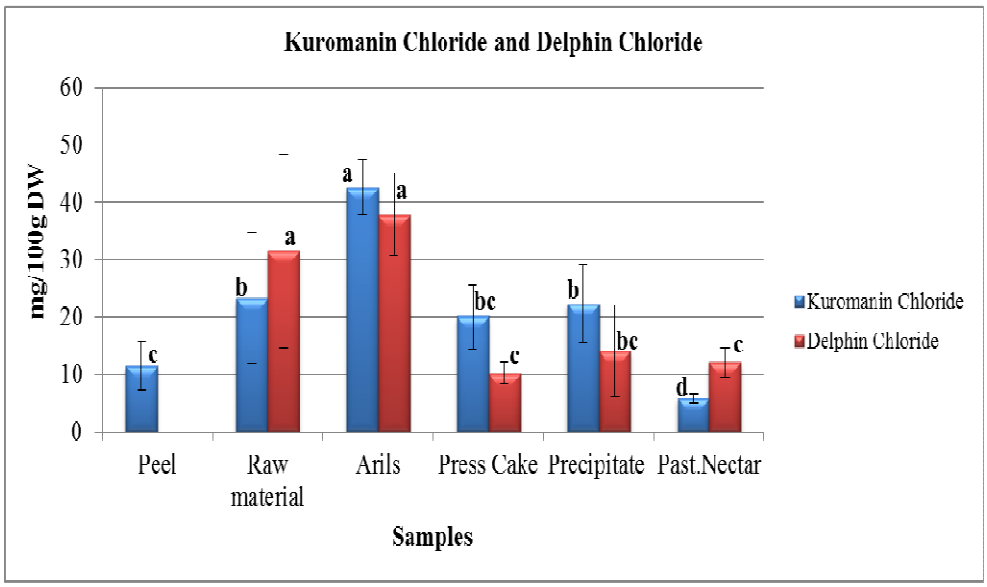


Figure 4.29: Evaluation of kuromanin chloride and delphin chloride concentration of raw materials, waste products and the product.

The anthocyanin content of some juices can increase after pasteurization, but after storage there can be a reduction. This was probably due to effect of two events as thermal applications effected extraction of anthocyanins previously, and then complexed or polymerized and retention of active principles caused by the inactivation of enzymes in their catabolism (Alighourchi et al., 2008). Lee et al. (2002) reported that pasteurized blackberry juice had higher amount of anthocyanins than initial juice and results found in our study are consistent with them.

Turfan et al. (2008) determined anthocyanin profile of pomegranate juice by HPLC and they detected cyn 3,5-dOg (56%) as major anthocyanin and others cyn 3-O-glu (25%), del 3,5-dOg (9%), del 3-O-glu (4%), pel 3,5-dOg (3%) and pel 3-O-glu (2%). They also reported that during pasteurization there were 15% losses of cyn 3-O-glu and 16 of cyn 3,5-dOg. Clarification did not change cyn 3,5-dOg concentration, but caused 10% losses of cyn 3-O-glu. In this study, clarification did not cause any important change in anthocyanin composition; however, pasteurization increased only cyn 3,5-dOg concentration.

In the study of Ozkal & Dinc (1993), chemical composition and biological activites of pomegranate were reviewed. According to their review, when partly purificated pomegranate peel extract and pomegranate arils were compared, it was observed that pel 3,5-dOg and pel 3-O-glu were present in high values in peel, and in low amounts in arils. In this study, pel 3,5-dOg could not be found in peel, but pel 3-O-glu was

only detected in raw material and peel. They also claimed that both of cyn 3,5-dOg and cyn 3-O-glu were detected in pomegranate peel, but del 3,5-dOg and del 3-O-glu which were major anthocyanins of pomegranate juice could not be detected in pomegranate peel. In this research, cyn 3-O-glu could not be found for raw material, arils and peel, however, cyn 3-O-glu was detected for each and arils were higher than others. Del 3-O-glu and del 3,5-dOg were also could not be detected for pomegranate peel as reported by Ozkal & Dinc (1993).

Mullen et al. (2007) evaluated phenolic compounds in fruit juices including pomegranate juice. They investigated cyanidin glucosides, delphinidin glucosides and quercetin. They only detected cyn 3-O-glu and q-3-O-glucoside. This was consistent with results in this study. Del 3,5-dOg could not be detected in peel, but in others. Cyn 3-O-glu and q-3-BDg were detected in all samples but cyn 3,5-dOg was not.

There are also some studies in which more anthocyanins of pomegranate juice were detected. Gil et al. (2000) detected del 3,5-dOg, cyn 3,5-dOg, cyn 3-glu, pel 3-O-glu and del 3-O-glu in commercial juices, commercial juice from concentrate, juice from fresh or frozen arils and single-strength commercial juice. Anthocyanin concentration can be degraded or reduced during storage and depends on the growth conditions (Ozkal & Dinc, 1993).

4.8 Results of Potential Bioavailability Analysis by *In Vitro* Digestion Method

4.8.1 Results of total phenolic content analysis of potential bioavailability

Standard calibration curve of gallic acid was prepared as shown in Figure 4.30.

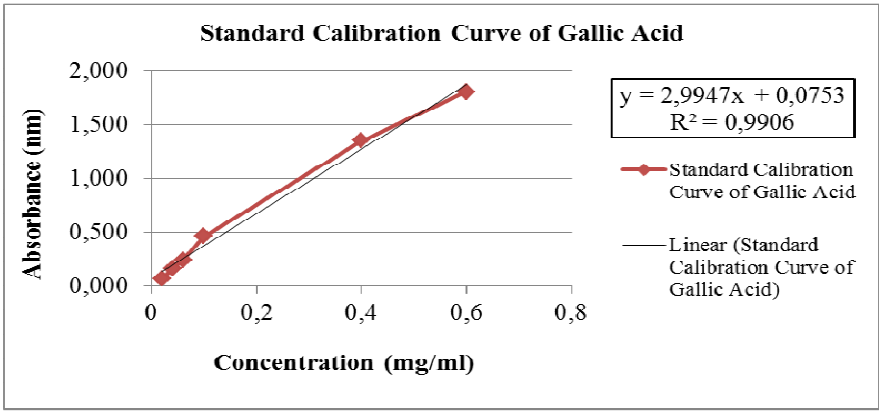


Figure 4.30: Standard calibration curve of gallic acid for potential bioavailability.

Results of total phenolic content analysis were expressed as mg GAE/100 g DW for each sample. The standard calibration curve was prepared between 0.02-0.6 mg/ml and the equation was used to evaluate the absorbance values of the samples measured by spectrophotometer.

Results of total phenolic content analysis of bioavailability samples are given in Table 4.12 and Figure 4.31. The results (PG, IN and OUT) were compared with methanolic extracts.

Table 4.12: Results for phenolic content at PG, IN and OUT fractions.

Sample	Extract	PG	IN	OUT
PP	18029.17 ± 1568.87 a	13526.71 ± 2226.44 a	1782.86 ± 595.08 a	20242.26 ± 1068.24 a
PA	5477.35 ± 972.94 b	5404.05 ±1228.13 b	1015.15 ± 117.36 b	5414.69 ± 1550.94 b
CON	3631.30 ± 217.27 c	3569.31 ± 67.09 cd	1689.17 ± 302.73 a	3719.04 ± 250.69 b
PN	1005.30 ± 115.50 d	657.74 ± 378.37 e	196.56 ± 11.35 c	198.05 ± 319.13 c
PAC	3318.81 ± 501.29 c	5020.99 ± 906.43 bc	1010.30 ± 168.92 b	5173.63 ± 2325.94 b
PC	3171.30 ± 390.90 c	2389.45 ± 634.85 d	480.43 ± 81.56 c	4760.35 ± 771.04 b

As showed in Figure 4.31, PG, IN and OUT values of peel were the highest and pasteurized nectar was the lowest. There was no difference between PG of arils and precipitate; concentrate and press cake. According to IN fraction value which is available in serum, peel showed highest bioavailability due to including high phenolic content. IN fraction of peel and concentrate; arils and precipitate; press cake and pasteurized nectar were same in between.

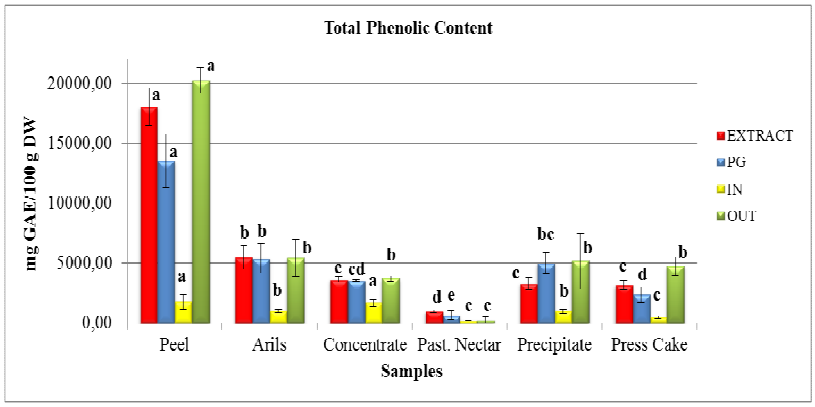


Figure 4.31: Results for total phenolic content at each fraction.

There was no significant difference between OUT fraction of arils, concentrate, precipitate and press cake. Most phenolics are discarded as by products or wastes with bioavailability equal to that of arils. Very little bioavailability was obtained for final product.

Extract was accepted as 100% and total phenolic content % residues of PG, IN and OUT fractions for each sample were also calculated in Table 4.13. PG% was high for arils and concentrate; however % residue of concentrate was higher than arils. Although peel showed the greatest amount as mg GAE/100 g DW for extract, IN, PG and OUT, it had the lowest % residue from extract. IN value of concentrate was closer to peel, but due to higher % residue, it can be said that concentrate showed a better bioavailable potential than peel for total phenolic.

Table 4.13: Total phenolic content % residues of PG, IN and OUT.

Sample	PP	PA	CON	PN	PAC	PC
Extract	100	100	100	100	100	100
PG	75.03	98.66	98.29	65.43	151.29	75.35
IN	9.89	18.53	46.52	19.55	30.44	15.15
OUT	112.28	98.86	102.42	19.70	155.89	150.11

4.8.2 Results for potential total anthocyanin bioavailability

Results of total anthocyanin content analysis of samples were expressed as mg cyn-3-gly/100 g DW. OUT of press cake was too low and close to zero, so it was ignored for statistical evaluation. Results for total anthocyanin bioavailability in samples and comparison with methanolic extracts are shown in Table 4.14 and Figure 4.32.

Table 4.14: Results of total anthocyanin content analysis of PG, IN and OUT.

Sample	Extract	PG	IN	OUT
PP	52.60 ± 11.77 d	29.87 ± 13.11 c	0.94 ± 1.16 a	16.73 ± 14.40 ab
PA	176.60 ± 25.00 a	129.13 ± 23.68 a	3.54 ± 1.78 a	26.72 ± 21.69 ab
CON	101.00 ± 310.86 c	86.21 ± 3.94 b	3.78 ± 2.16 a	24.89 ± 2.91 ab
PN	26.30 ± 3.30 e	4.41 ± 2.49 d	1.21 ± 1.10 a	5.06 ± 3.25 b
PAC	126.70 ± 3.13 b	71.44 ± 16.80 b	3.91 ± 3.36 a	44.91 ± 19.96 a
PC	48.40 ± 9.04 d	21.86 ± 13.20 c	1.38 ± 1.33 a	-

As presented in Figure 4.32, arils showed significantly higher PG than others and pasteurized nectar had the lowest.

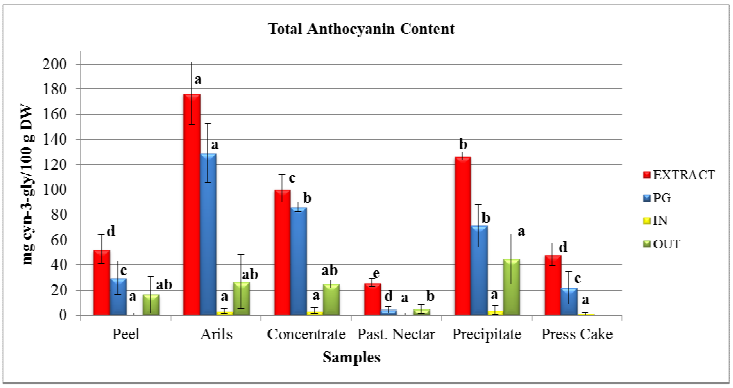


Figure 4.32: Results for potential anthocyanin bioavailability.

No difference was observed between PG of peel and press cake; also between concentrate and precipitate.

IN values were lower than OUT and for OUT and IN of all samples, there was not any difference. Total anthocyanin content % residues of PG, IN and OUT for each sample were evaluated in Table 4.15. IN % for all samples was low and closer to each other as calculated mg cyn-3-gly/100 g DW values. Potential anthocyanin bioavailability was at very low levels and did not create any difference for samples.

Table 4.15: Total anthocyanin content % residues of PG, IN and OUT.

Sample	PP	PA	CON	PN	PAC	PC
Extract	100	100	100	100	100	100
PG	56.79	73.12	86.14	16.77	56.38	45.16
IN	1.79	2.00	3.74	4.61	3.08	2.86
OUT	31.81	15.13	24.64	19.25	35.44	

4.8.3 Results for potential bioavailability by means of testing antioxidant activity

Standard calibration curve of Trolox showed in Figure 4.33 was prepared. Results of total antioxidant analysis by DPPH were expressed as mg TEAC/100 g DW for each sample. The standard calibration curve was prepared between 0.005-0.2 mg/ml and the equation was used to calculate the absorbance values of the samples measured by spectrophotometer.

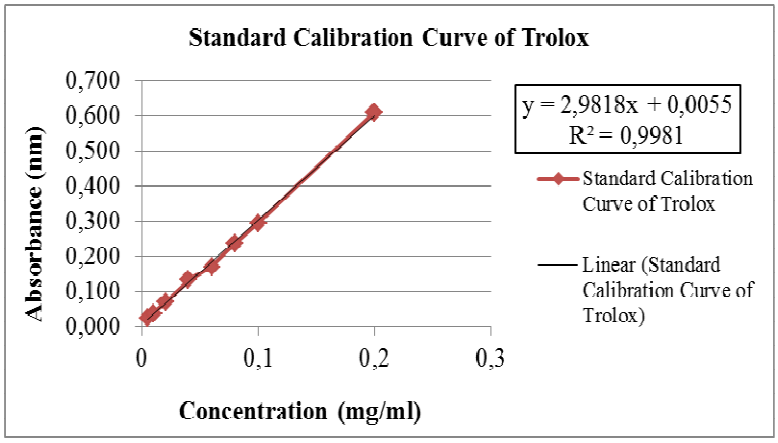


Figure 4.33: Standard calibration curve of Trolox for DPPH of potential bioavailability.

Results for total antioxidant activity in bioavailability samples are shown in Table 4.16 and Figure 4.34.

Table 4.16: Results for total antioxidant activity by DPPH method at PG, IN and OUT fractions.

Sample	Extract	PG	IN	OUT
PP	42884.98 ± 5798.35 a	2332.29 ± 168.37 a	19.84 ± 9.90 c	1877.33 ± 379.84 a
PA	11829.58 ± 3087.95 b	2634.97 ± 536.63 a	129.82 ± 28.02 a	833.05 ± 594.50 b
CON	7040.30 ± 945.35 c	1012.57 ± 70.34 b	33.83 ± 2.41 bc	727.06 ± 140.38 bc
PN	1843.50 ± 246.62 d	1567.21 ± 898.17 b	66.34 ± 43.88 b	296.05 ± 162.44 c
PAC	6865.50 ± 875.86 c	2337.75 ± 292.42 a	122.77 ± 18.27 a	2011.91 ± 30.99 a
PC	6147.40 ± 591.74 c	895.59 ± 324.01 b	44.56 ± 9.34 bc	591.51 ± 166.53 bc

According to Figure 4.34, PG values of peel, arils and precipitate were same, however arils and precipitate showed closer and higher IN values. IN of peel was the lowest and it was same with concentrate and press cake. OUT of peel and precipitate; arils, concentrate and press cake; concentrate, press cake and pasteurized nectar were not different.

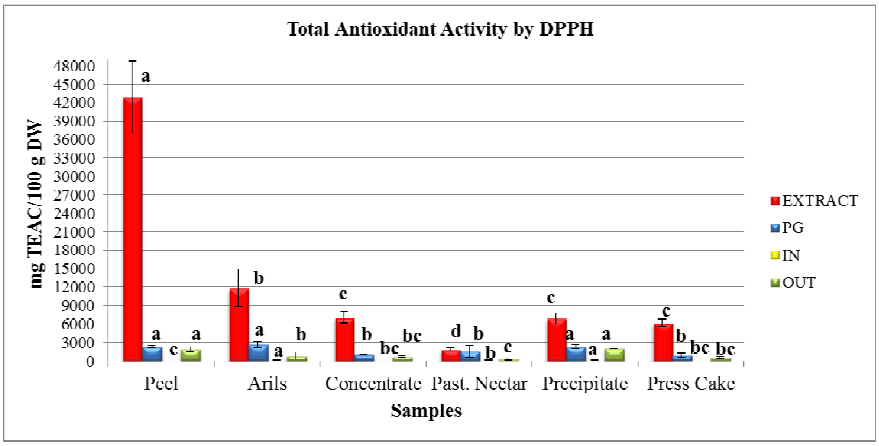


Figure 4.34: Results of total antioxidant activity in bioavailability samples.

Total antioxidant activity % residue by DPPH residues of PG, IN and OUT for each sample are presented in Table 4.17. IN % values of all samples were not high and different from each other. PG of pasteurized nectar, precipitate and concentrate was high, respectively. Although concentrate showed higher total phenolics bioavailability, it had low total antioxidant analysis by DPPH. It is clear that for precipitate significantly higher amounts of antioxidant acitivity in PG, IN and OUT fractions were found which the discarded fraction is.

Table 4.17: DPPH analysis % residues of PG, IN and OUT.

Sample	PP	PA	CON	PN	PAC	PC
Extract	100.00	100.00	100.00	100.00	100.00	100.00
PG	5.44	22.27	14.38	85.01	34.05	14.57
IN	0.05	1.10	0.48	3.60	1.79	0.72
OUT	4.38	7.04	10.33	16.06	29.30	9.62

Perez-Vicente et al. (2002) studied the same *in vitro* digestion method to determine bioavailability of phenolic compounds and anthocyanins. According to total phenolic content analysis, pepsin digestion did not change the values, however, 29% of total phenolic compounds were detected in IN after pancreatin-bile salt digestion. The same results were found for arils and precipitate in this study and for arils 98% of total phenolic were present in PG, so there was no important loss.

For anthocyanin bioavailability, Perez-Vicente et al. reported that % residue in IN and % residue in OUT were 2.4 and 15.3, respectively. These values were also closer to the ones detected in this study. Therefore, the anthocyanin and total phenolic results were consistent with Perez-Vicente's study.

McDougall et al. (2005) assessed potential bioavailability of raspberry anthocyanins by using the same system and reported that only 5.3% of total anthocyanins and 10.3 of total phenolics were recovered after digestion. Some of pomegranate samples in this study showed higher % total phenolic residues and closer % total anthocyanin residues.

Fazari et al. (2008) determined increase in PG for total phenolics and not any change for anthocyanins. In IN, total phenolics were about 26-30% and anthocyanins were 15-21% for frozen sweet cherries by using *in vitro* bioavailability. According to the results in this study, bioavailability of total phenolics of pomegranate arils was closer to frozen cherries, whereas bioavailability of anthocyanins of frozen cherries was better than pomegranate's, significantly.

The reason of the high loss of anthocyanins is not completely known but there can be some factors such as conversions of anthocyanins. They are metabolized to noncolored forms, oxidized or degraded into other chemicals which cannot be detected under these conditions. It is also important that no aglycons occur after pancreatin bile (intestinal) digestion (Perez-Vicente et al., 2002).

It could be due to release of phenolic compounds from matrix after pepsin digestion. It was dependent on pH conditions. At low pH, anthocyanins occur in the red form of flavylium cation (Fazzari et al, 2008).

On the other hand, comparison of *in vitro* studies are not easy because of differences starting from the original material to the *in vitro* digestion procedure applied (Fazzari et al, 2008).

4.8.4 Results of major phenolic compounds and anthocyanin analysis by HPLC-PDA for potential bioavailability

Gallic acid, ferulic acid, catechin, q-3-g, q-3-BDg were usually detected in extracts, PG and some of IN and OUT of bioavailability samples.

Cyn 3-O-glu, del 3,5-dOg, pel 3,5-dOg and pel 3-dOg could not be detected for IN and OUT for all samples, so anthocyanin bioavailability was very low.

p-coumaric acid could not be found in any samples, but it had been detected in extract of peel before.

Neochlorogenic acid was only found in PG of peel as 480.67 ± 51.57 mg/100g DW.

According to results of major compound analysis by HPLC-PDA for PG, IN, OUT of bioavailability samples as presented in Table 4.18; peel had the highest values of PG, IN and OUT of gallic acid, catechin, ferulic acid, q-3-g and q-3-BDg concentrations due to also showing the highest values for extracts. IN values were lower than OUT for all samples. Gallic acid was only detected for IN of peel and arils. There was no catechin in any IN sample and peel showed the only OUT value.

Major anthocyanin analysis for bioavailability samples was performed by HPLC and evaluated as shown in Table 4.19. PG of cyn 3-O-glu was the highest for arils as observed for extract. There was no difference between PG of peel, concentrate, precipitate; pasteurized nectar and press cake for cyn 3-O-glu. Del 3,5-dOg was not detected in PG and extract of peel. PG of arils and concentrate; pasteurized nectar, press cake, precipitate were same for del 3,5-dOg, statistically. PG of pel 3,5-dOg was the highest for arils and it was not found in PG of peel. Pel 3-O-glu was not detected for PG of pasteurized nectar. Peel showed the highest PG of pel 3-O-glu. Pel 3,5-dOg for pasteurized nectar and pel 3-O-glu for arils, concentrate, precipitate and press cake was detected for PG samples, although they were not found for extracts. This was probably due to effect of digestion enzymes and there might be some changes or conversions for anthocyanins during digestion.

Generally, there was an increase in PG for phenolic compounds and anthocyanins, however excessive decrease in IN and OUT for anthocyanins. This was probably due to enhancer effect of enzymes such as pepsin and acidic pH of the medium in gastric

conditions and effect of pancreatin-bile mixtures and alkaline pH of the medium in intestinal conditions during *in vitro* digestion (Bermudez-Soto et al., 2007).

Table 4.18: Major phenolic compound analysis by HPLC of bioavailability samples.

Samples	**Gallic Acid (mg/100g DW)**			
	Extract	**PG**	**IN**	**OUT**
PP	417.67 ± 355.43 a	1890.51 ± 1267.13 a	52.01 ± 31.16	1126.55 ± 368.82 a
PA	142.59 ± 28.93 b	560.66 ± 127.70 b	28.04 ± 5.74	38.70 ± 14.74 b
CON	100.41 ± 10.43 b	327.48 ± 61.82 b	ND	57.72 ± 21.50 b
PN	26.10 ± 2.33 b	121.33 ± 11.42 b	ND	ND
PAC	65.91 ± 9.34 b	297.87 ± 16.18 b	ND	45.77 b
PC	54.63 ± 19.11 b	157.65 ± 91.25 b	ND	118.41 ± 45.42 b
Samples	**Catechin (mg/100g DW)**			
	Extract	**PG**	**IN**	**OUT**
PP	8101.31 ± 2078.26 a	21405.93 ± 12381.92 a	ND	16104.78 ± 9691.39
PA	506.48 ± 96.47 b	842.41 ± 269.38 b	ND	ND
CON	ND	ND	ND	ND
PN	10.47 b	22.84 b	ND	ND
PAC	ND	72.80 ± 26.25 b	ND	ND
PC	289.55 ± 89.77 b	96.14 ± 11.37 b	ND	ND
Samples	**Ferulic Acid (mg/100g DW)**			
	Extract	**PG**	**IN**	**OUT**
PP	54.94 ± 18.17 a	426.59 ± 77.65 a	68.66 ± 25.19 a	377.944 ± 130.30 a
PA	47.56 ± 4.23 a	157.36 ± 39.63 b	11.04 ± 3.36 bc	48.20 ± 14.37 bc
CON	28.84 ± 8.29 b	99.33 ± 18.99 c	5.72 ± 0.66 c	17.23 ± 8.96 bc
PN	5.84 ± 0.58 c	23.83 ± 4.00 d	2.83 ± 0.82 c	4.42 ± 1.30 c
PAC	20.56 ± 1.92 b	97.24 ± 14.85c	30.60 ± 13.60 b	94.62 ± 52.53 b
PC	24.88 ± 4.98 b	63.02 ± 22.02 cd	8.80 ± 1.31 c	55.70 ± 7.04 bc
Samples	**Q-3-g (mg/100g DW)**			
	Extract	**PG**	**IN**	**OUT**
PP	117.15 ± 12.41 a	499.68 ± 116.98 a	71.44 ± 33.13 a	428.77 ± 124.89 a
PA	9.14 b	25.58 ± 19.07 b	7.17 ± 2.28 b	28.27 ± 18.55 b
CON	4.88 ± 0.11 b	5.46 b	3.13 b	9.77 b
PN	ND	3.12 b	ND	ND
PAC	ND	15.77 ± 3.12 b	14.20 ± 8.03 b	45.64 ± 23.03 b
PC	15.28 ± 3.33 b	20.22 ± 8.66 b	3.38 ± 1.26 b	3.38 ± 1.26 b
Samples	**Q-3-BDg (mg/100g DW)**			
	Extract	**PG**	**IN**	**OUT**
PP	447.07 ± 311.11 a	1954.98 ± 917.24 a	347.30 ± 197.15 a	3233.11 ± 882.55 a
PA	264.15 ± 74.40 ab	391.65 ± 240.63 b	78.46 ± 27.08 b	336.07 ± 20.83 b
CON	67.41 ± 26.80 bc	284.96 ± 109.78 b	45.71 ± 7.55 b	290.33 ± 87.82 b
PN	18.26 ± 5.60 c	79.22 ± 14.11 b	24.93 ± 8.07 b	51.28 ± 15.13 b
PAC	98.27 ± 21.55 bc	183.29 ± 19.95 b	79.44 ± 33.13 b	390.12 ± 169.94 b
PC	105.86 ± 21.60 bc	137.26 ± 64.10 b	22.33 ± 2.37 b	356.54 ± 39.50 b

(ND: Not Determined)

During pepsin digestion, phenolic compounds are released from the food matrix (Fazzari et al, 2008). As reported by Perez-Vicente et al., the reason of the important loss of anthocyanins is not known and there can be some conversions of anthocyanins to such as noncolored forms, oxidizations or degradations (Perez-Vicente et al., 2002).

Results of major anthocyanin analysis by HPLC of bioavailability samples are presented in Table 4.19.

Table 4.19: Major anthocyanin analysis by HPLC of bioavailability samples.

Samples	**Kuromanin Chloride (Cyn 3-O-Glu) (mg/100g DW)**			
	Extract	**PG**	**IN**	**OUT**
PP	11,51 ± 4,34 c	99.69 ± 21.55 b	ND	ND
PA	42,63 ± 4,86 a	170.47 ± 25.00 a	ND	ND
CON	21,03 ± 0,22 b	96.47 ± 10.90 b	ND	ND
PN	5,85 ± 0,84 c	25.97 ± 1.12 c	ND	ND
PAC	22,38 ± 6,91 b	92.71 ± 20.40 b	ND	ND
PC	20,13 ± 5,58 b	49.51 ± 16.80 c	ND	ND
	Delphin Chloride (Del 3,5-dOg) (mg/100g DW)			
Samples	**Extract**	**PG**	**IN**	**OUT**
PP	ND	ND	ND	ND
PA	37,86 ± 7,19 a	83.70 ± 41.38 ab	ND	ND
CON	35,56 ± 16,34 ab	105.55 83.70 ± 52.98 a	ND	ND
PN	12,09 ± 2,50 bc	38.81 83.70 ± 9.02 b	ND	ND
PAC	14,19 ± 8,00 bc	49.42 83.70 ± 20.58 b	ND	ND
PC	10,26 ± 1,85 c	37.33 83.70 ± 11,47 b	ND	ND
Samples	**Pelargonin Chloride (Pel 3,5-dOg) (mg/100g DW)**			
	Extract	**PG**	**IN**	**OUT**
PP	ND	ND	ND	ND
PA	103,48 ± 50,44 a	401.23 ± 149.53 a	ND	ND
CON	38,28 ± 3,69 b	166.21 ± 23.97 b	ND	ND
PN	ND	28.29 c	ND	ND
PAC	17,31 ± 5,29 b	133.24 ± 44.56 bc	ND	ND
PC	17,48 ± 5,83 b	34.07 ± 12.85 bc	ND	ND
Samples	**Callistephin Chloride (Pel 3-O-glu) (mg/100g DW)**			
	Extract	**PG**	**IN**	**OUT**
PP	12,42 ± 0,77	57.19 ± 7.65 a	ND	ND
PA	ND	19.68 ± 6.40 b	ND	ND
CON	ND	11.24 ± 1.41 c	ND	ND
PN	ND	ND	ND	ND
PAC	ND	11.72 ± 4.13 c	ND	ND
PC	ND	5.45 ± 2.67 c	ND	ND

(ND: Not Determined)

4.9 The Relations between Total Phenolic, Total Flavonoid, Total Anthocyanin, Total Tannin Contents and Total Antioxidant Activity Methods

The relations between all of total antioxidant activity methods (CUPRAC, DPPH, FRAP and ABTS) and total phenolic, total flavonoid, total anthocyanin and total tannin contents were evaluated by basic linear regression analysis and SPSS. The correlation coefficents were showed in Table 4.20 and ANOVA table of regression variance analysis were presented in APPENDIX A in Table A.11-A.17.

Table 4.20: The relation between total phenolic content, total flavonoid content, total anthocyanin content, total tannin content and total antioxidant activity methods.

Methods	Total Phenolic Content	Total Flavonoid Content	Total Anthocyanin Content	Total Tannin Content	CUPRAC	DPPH	FRAP	ABTS
CUPRAC	0.968 a	0.972 a	0.045	0.900 a	-	-	-	-
DPPH	0.986 a	0.995 a	0.001	0.871 a	0.986 a	-	-	-
ABTS	0.976 a	0.991 a	0.033	0.844 a	0.964 a	0.990 a	0.990 a	-
FRAP	0.987 a	0.991 a	0.027	0.844 a	0.970 a	0.994 a	-	-

(a: The regression variance analysis result is significant, statistically ($p<0.05$).)

According to Table 4.20, there was an important relation between total phenolic content and all of total antioxidant activity methods, CUPRAC ($r=0.968$), DPPH (0.986), ABTS (0.976) and FRAP (0.987), ($p<0.05$). The relation between total flavonoid content and all total antioxidant methods; and the relation between total tannin content and all total antioxidant methods were also determined as significant, statistically ($p<0.05$). However, the correlation between total anthocyanin content and total antioxidant activity methods was not important, statistically ($p>0.05$). There was an important relation between CUPRAC and DPPH, ABTS and FRAP, statistically and the correlation coefficents were determined as 0.986, 0.964 and 0.970, respectively. Moreover, the correlations between DPPH and ABTS ($r=0.990$); DPPH and FRAP (0.994); and FRAP and ABTS ($r=0.990$) were also important ($p<0.05$). A linear relation was determined between total antioxidant activity methods during pasteurized pomegranate nectar processing. Elfalleh et al. determined high correlation between total phenolic content and antioxidant activity of pomegranate extract (2002). In this study, total phenolic, total flavonoid, and total tannin contents also showed a significant linear relation with total antioxidant activity methods. However, total anthocyanin content by pH differential method did not show any linearity with any total antioxidant activity methods.

5. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the originality of this master thesis is coming from the evaluation of all processing steps of pomegranate concentrate and nectar production in the industrial scale by means of changes in polyphenols and antioxidant activity. By the data and knowledge obtained throughout this research hopefully it has been a useful study for the industry. For industrial pasteurized pomegranate nectar production samples including all of the products and processing steps; total phenolic content, total flavonoid content, total anthocyanin content, total tannin content, polymeric color (%), total antioxidant activity, major phenolic compounds profile by HPLC analysis were completed successfully. Moreover, *in vitro* digestion was performed for peel, raw material, arils, concentrate, precipitate after clarification, press cake and the final product, pasteurized pomegranate nectar. Total phenolic content, total anthocyanin content and total antioxidant activity by DPPH analysis were carried out for bioavailability samples. Changes and losses during the production were monitored by evaluating all processing steps; and also by comparing raw material, arils, waste products and the final product, separately.

Pomegranate showed significantly high phenolic content and antioxidant activity and raw material was found to be a good source of antioxidants and phenolics, which had high values due to including of peel.

When the results were evaluated by means of changes during production (from raw material to the product), it was seen that raw material showed the highest total phenolic, total flavonoid, total tannin contents and total antioxidant activity. On the other hand, cooling had the highest levels of total anthocyanin content and generally there was an important reduction trend from raw material to mashing, except for the total anthocyanin content because peel did not show important anthocyanin content.

Total antioxidant activity by CUPRAC, total tannin and total phenolic contents did not change significantly from mashing to evaporation during production. For total antioxidant activity by ABTS, FRAP and DPPH, there were effects of heating, however, it was important for only ABTS. An increase was observed during heating

for total antioxidant activity by CUPRAC, but it was not important significantly. Thermal treatments showed no effect on total phenolic content for production of juice and nectar. However, pasteurization caused significant decrease for total flavonoid content. There was no decrease in total anthocyanin content from raw material to mashing. Besides production of nectar, ultrafiltration and concentration also caused a decrease when they were compared to raw material. For total tannin content, there was a decrease during peeling and an increase during pasteurization, but this was not important, statistically.

According to the comparison of the product, raw materials, waste products, it was observed that final product, pasteurized nectar, had the lowest values for all analyses due to increase in glucose concentration. However, pasteurization of nectar during processing did not cause significant changes.

Press cake and precipitate showed similar results for all analyses statistically, except for total anthocyanin, because precipitate showed higher values than press cake. Total antioxidant activity by FRAP, CUPRAC and DPPH showed that precipitate and press cake had high values as arils. Precipitate showed high anthocyanin content as raw material. High amouts of tannin, flavonoid and anthocyanin was discarded by waste products.

The correlation between total phenolic, anthocyanin, flavonoid and tannin contents and total antioxidant activity methods were also evaluated statistically. There was a significant relation between total antioxidant activity methods and all of total compound content analysis, except anthocyanin.

Pasteurization showed the highest polymeric color (%) values, whereas cooling showed the lowest and this verified that polymeric color increased during heat treatment. No significant difference was observed between raw material, arils, product and waste products.

Gallic acid, ferulic acid, q-3-BDg, del 3,5-dOg and cyn 3-O-glu were detected as major phenolics and quantified for all samples by RP-HPLC/DAD analyses. Peel showed the highest gallic acid and q-3-BDg concentration, which were significantly different from other samples. After pressing, cooling and mashing; there was an increase for gallic acid concentration. Q-3-BDg also showed an increase after mashing. Raw material had the highest ferulic acid concentration. Cooling showed

the highest del 3,5-dOg and pasteurization had the highest cyn 3-O-glu concentrations. Pel 3-O-glu was only detected in peel and raw material at low amounts.

From potential bioavailability evaluation; PG, IN and OUT values of peel were highest in total phenolic content, but IN % residue values for total antioxidant activity were the lowest. IN % residue value of arils for total anthocyanin content and IN % residue value of pasteurized nectar for DPPH were higher than other samples. IN values of all samples were very low when they were compared to extract values. When potential bioavailabilities of phenolics were evaluated by means of processes, peels with their highest levels of phenolics were significantly preserved during digestion. Concentrated juice and by products such as precipitate and press cake had some preserved levels of phenolics, but pasteurized nectar as the end product significantly lost its phenolics during digestion. Heat applications, evaporation and pasteurization were found to be negatively affecting the antioxidant activity after digestion. When potential bioavailability of anthocyanins were evaluated, highest value was obtained for arils; however the highest preserved levels during digestion was obtained for concentrate and pasteurized nectar, especially, after gastric conditions and in serum. Waste products also possessed anthocyanins with about 50% bioavailability after gastric conditions and 2-3% in the serum fraction.

Phenolic acid and anthocyanin concentrations increased after postgastric conditions due to effect of acidic pH and enzymes, however, the concentrations in IN very low and anthocyanins could not enter in the serum (IN fraction). Potential anthocyanin bioavailability was found to be much lower than that of potential phenolic bioavailability. Conditions of processing steps such as mashing and pasteurization can be optimized to protect health effects of pomegranate by means of phenolic content, flavonoid content, tannin content and antioxidant activity. Waste products, especially, pomegranate peel can be used as a functional ingredient in dietary supplements or food formulations. Waste products can gain economic value by using in different products and health effects of pomegranate can be extended to different food product formulations.

As the future study, for potential bioavailability evaluation besides analysis of total phenolic content and total anthocyanin content, it is recommended to analyze total flavonoid content and total tannin content. Total antioxidant activity should also be

researched by using other methods such as ABTS, CUPRAC and FRAP. Moreover, bioavailability should be supported by *in vivo* studies. This study showed rich polyphenol content and antioxidant activity of pomegranate, but the effect of consumption of pomegranate with different foods such as including high fat, sugar or protein content on bioavailability should also be investigated to identify the mechanisms underlying bioavailability results.

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APPENDICES

APPENDIX A: ANOVA Tables
APPENDIX B: Calibration Curves of Standards for HPLC
APPENDIX C: HPLC Chromatograms

APPENDIX A

Table A.1: Each analysis for all samples.

Analysis		Sum of Squares	df	Mean Square	F	Sig.
Total Phenolic Content	Between Groups	9.847E8	14	7.033E7	56.511	.000
	Within Groups	5.352E7	43	1244619.878		
	Total	1.038E9	57			
CUPRAC	Between Groups	3.017E10	14	2.155E9	41.990	.000
	Within Groups	2.207E9	43	5.132E7		
	Total	3.238E10	57			
DPPH	Between Groups	6.138E9	14	4.384E8	58.873	.000
	Within Groups	3.202E8	43	7446781.972		
	Total	6.458E9	57			
Total Flavonoid Content	Between Groups	1.798E9	14	1.284E8	47.829	.000
	Within Groups	1.155E8	43	2685396.934		
	Total	1.914E9	57			
FRAP	Between Groups	2.101E9	14	1.501E8	42.053	.000
	Within Groups	1.535E8	43	3568775.270		
	Total	2.255E9	57			
ABTS	Between Groups	9.225E9	14	6.589E8	161.457	.000
	Within Groups	1.755E8	43	4081210.655		
	Total	9.401E9	57			
Total Anthocyanin Content	Between Groups	129990.431	14	9285.031	19.819	.000
	Within Groups	20145.633	43	468.503		
	Total	150136.064	57			
Total Tannin Content	Between Groups	1.318E7	14	941632.267	17.353	.000
	Within Groups	2333297.791	43	54262.739		
	Total	1.552E7	57			
Polymeric Color (%)	Between Groups	5235.137	13	402.703	2.466	.014
	Within Groups	6531.275	40	163.282		
	Total	11766.412	53			

Table A.2: Each analysis for process steps.

Analysis		Sum of Squares	df	Mean Square	F	Sig.
Total Phenolic Content	Between Groups	2.836E8	10	2.836E7	20.894	.000
	Within Groups	4.208E7	31	1357498.646		
	Total	3.257E8	41			
CUPRAC	Between Groups	9.566E9	10	9.566E8	18.717	.000
	Within Groups	1.584E9	31	5.111E7		
	Total	1.115E10	41			
DPPH	Between Groups	1.670E9	10	1.670E8	27.631	.000
	Within Groups	1.874E8	31	6044857.138		
	Total	1.858E9	41			
Total Flavonoid Content	Between Groups	5.111E8	10	5.111E7	22.072	.000
	Within Groups	7.178E7	31	2315413.663		
	Total	5.828E8	41			
FRAP	Between Groups	5.420E8	10	5.420E7	17.485	.000
	Within Groups	9.610E7	31	3099868.297		
	Total	6.381E8	41			
Total Ant. Content	Between Groups	82527.522	10	8252.752	14.550	.000
	Within Groups	17583.586	31	567.212		
	Total	100111.108	41			
ABTS	Between Groups	2.464E9	10	2.464E8	63.603	.000
	Within Groups	1.201E8	31	3873762.184		
	Total	2.584E9	41			
Total Tannin Content	Between Groups	9267534.220	10	926753.422	18.944	.000
	Within Groups	1516508.401	31	48919.626		
	Total	1.078E7	41			
Polymeric Color (%)	Between Groups	4462.996	9	495.888	3.050	.011
	Within Groups	4553.115	28	162.611		
	Total	9016.111	37			

Table A.3: Each analysis for RM, PA, waste products and the product.

Analysis		Sum of Squares	df	Mean Square	F	Sig.
Total Phenolic Content	Between Groups	8.217E8	5	1.643E8	68.661	.000
	Within Groups	4.308E7	18	2393439.407		
	Total	8.648E8	23			
Total Flavonoid Content	Between Groups	1.394E9	5	2.788E8	59.115	.000
	Within Groups	8.488E7	18	4715509.420		
	Total	1.479E9	23			
Total Anthocyanin Content	Between Groups	47270.889	5	9454.178	51.019	.000
	Within Groups	3335.505	18	185.306		
	Total	50606.394	23			
Total Tannin Content	Between Groups	9505424.799	5	1901084.960	17.460	.000
	Within Groups	1959865.960	18	108881.442		
	Total	1.147E7	23			
CUPRAC	Between Groups	2.258E10	5	4.517E9	43.681	.000
	Within Groups	1.861E9	18	1.034E8		
	Total	2.444E10	23			
DPPH	Between Groups	4.911E9	5	9.822E8	65.794	.000
	Within Groups	2.687E8	18	1.493E7		
	Total	5.180E9	23			
ABTS	Between Groups	7.656E9	5	1.531E9	199.548	.000
	Within Groups	1.381E8	18	7673388.918		
	Total	7.794E9	23			
FRAP	Between Groups	1.747E9	5	3.494E8	56.168	.000
	Within Groups	1.120E8	18	6220941.586		
	Total	1.859E9	23			
Polymeric Color (%)	Between Groups	873.167	5	174.633	1.107	.395
	Within Groups	2524.819	16	157.801		
	Total	3397.986	21			

Table A.4: Phenolic and anthocyanin analysis by HPLC for all samples.

Compound		Sum of Squares	df	Mean Square	F	Sig.
Catechin	Between Groups	2.026E8	7	2.895E7	35.782	.000
	Within Groups	1.375E7	17	808957.303		
	Total	2.164E8	24			
Gallic Acid	Between Groups	405401.199	14	28957.228	4.345	.000
	Within Groups	266550.671	40	6663.767		
	Total	671951.870	54			
Ferulic Acid	Between Groups	15506.627	14	1107.616	17.219	.000
	Within Groups	2380.000	37	64.324		
	Total	17886.627	51			
Q-3-g	Between Groups	38173.448	8	4771.681	8.660	.000
	Within Groups	11571.374	21	551.018		
	Total	49744.822	29			
Q 3,5-Bdg	Between Groups	721630.404	14	51545.029	5.590	.000
	Within Groups	350378.473	38	9220.486		
	Total	1072008.878	52			
Del 3,5-dOg	Between Groups	8258.343	13	635.257	7.397	.000
	Within Groups	2834.214	33	85.885		
	Total	11092.556	46			
Pel 3,5-dOg	Between Groups	51635.384	10	5163.538	1.866	.098
	Within Groups	71946.215	26	2767.162		
	Total	123581.598	36			
Cyn 3-O-glu	Between Groups	8437.180	14	602.656	5.735	.000
	Within Groups	4203.337	40	105.083		
	Total	12640.518	54			

Table A.5: Phenolic and anthocyanin analysis by HPLC for process steps.

Compound		Sum of Squares	df	Mean Square	F	Sig.
Gallic Acid	Between Groups	79619.776	10	7961.978	23.050	.000
	Within Groups	10017.362	29	345.426		
	Total	89637.139	39			
Ferulic Acid	Between Groups	12294.533	10	1229.453	20.949	.000
	Within Groups	1584.542	27	58.687		
	Total	13879.075	37			
Q 3,5-Bdg	Between Groups	238227.603	10	23822.760	15.252	.000
	Within Groups	40610.549	26	1561.944		
	Total	278838.152	36			
Del 3,5-dOg	Between Groups	5694.952	10	569.495	5.604	.000
	Within Groups	2540.657	25	101.626		
	Total	8235.609	35			
Cyn 3-O-glu	Between Groups	6321.290	10	632.129	4.610	.001
	Within Groups	3839.278	28	137.117		
	Total	10160.568	38			

Table A.6: HPLC analysis for RM, waste products and the product.

Compound		Sum of Squares	df	Mean Square	F	Sig.
Gallic Acid	Between Groups	358255.828	5	71651.166	4.746	.007
	Within Groups	256635.577	17	15096.210		
	Total	614891.405	22			
Ferulic Acid	Between Groups	12166.732	5	2433.346	25.532	.000
	Within Groups	1524.909	16	95.307		
	Total	13691.642	21			
Q 3,5-Bdg	Between Groups	514865.743	5	102973.149	5.625	.003
	Within Groups	311214.701	17	18306.747		
	Total	826080.444	22			
Del 3,5-dOg	Between Groups	2343.358	4	585.840	6.971	.003
	Within Groups	1176.539	14	84.038		
	Total	3519.897	18			
Cyn 3-O-glu	Between Groups	3175.441	5	635.088	17.196	.000
	Within Groups	627.834	17	36.931		
	Total	3803.275	22			

Table A.7: Extracts for total phenolic, total anthocyanin content and DPPH analysis.

Analysis		Sum of Squares	df	Mean Square	F	Sig.
Total Phenolic Content for Extracts	Between Groups	7.616E8	5	1.523E8	236.007	.000
	Within Groups	1.162E7	18	645440.281		
	Total	7.733E8	23			
DPPH for extracts	Between Groups	4.555E9	5	9.110E8	120.852	.000
	Within Groups	1.357E8	18	7538059.448		
	Total	4.691E9	23			
Total Anthocyanin Content for Extracts	Between Groups	64565.128	5	12913.026	79.019	.000
	Within Groups	2941.505	18	163.417		
	Total	67506.633	23			

Table A.8: PG, IN and OUT of bioavailability samples for analysis.

Analysis		Sum of Squares	df	Mean Square	F	Sig.
Total Phenolic Content for PG	Between Groups	4.021E8	5	8.043E7	61.568	.000
	Within Groups	2.351E7	18	1306276.413		
	Total	4.256E8	23			
Total Phenolic Content for IN	Between Groups	7994215.264	5	1598843.053	19.386	.000
	Within Groups	1484556.375	18	82475.354		
	Total	9478771.639	23			
Total Phenolic Content for OUT	Between Groups	9.689E8	5	1.938E8	119.667	.000
	Within Groups	2.915E7	18	1619290.087		
	Total	9.980E8	23			
DPPH for PG	Between Groups	1.105E7	5	2209443.711	10.055	.000
	Within Groups	3955434.841	18	219746.380		
	Total	1.500E7	23			
DPPH for IN	Between Groups	43388.367	5	8677.673	16.091	.000
	Within Groups	9707.367	18	539.298		
	Total	53095.734	23			
DPPH for OUT	Between Groups	1.016E7	5	2031635.249	21.292	.000
	Within Groups	1717531.698	18	95418.428		
	Total	1.188E7	23			
Total Anthocyanin Content for PG	Between Groups	41764.026	5	8352.805	43.303	.000
	Within Groups	3279.200	17	192.894		
	Total	45043.225	22			
Total Anthocyanin Content for IN	Between Groups	36.246	5	7.249	1.727	.185
	Within Groups	67.156	16	4.197		
	Total	103.402	21			
Total Anthocyanin Content for OUT	Between Groups	2931.957	4	732.989	1.558	.243
	Within Groups	6114.236	13	470.326		
	Total	9046.194	17			

Table A.9: Phenolic and anthocyanin analysis for extracts of bioavailability samples.

Compound		Sum of Squares	df	Mean Square	F	Sig.
Catechin	Between Groups	1.392E8	3	4.640E7	42.803	.000
	Within Groups	8671784.190	8	1083973.024		
	Total	1.479E8	11			
Gallic Acid	Between Groups	332855.160	5	66571.032	4.148	.013
	Within Groups	256767.073	16	16047.942		
	Total	589622.233	21			
Ferulic Acid	Between Groups	5812.010	5	1162.402	20.001	.000
	Within Groups	813.637	14	58.117		
	Total	6625.647	19			
Q-3-g	Between Groups		3	8601.398	201.637	.000
	Within Groups	341.263	8	42.658		
	Total	26145.457	11			
Q 3,5-BDg	Between Groups	503213.620	5	100642.724	5.496	.003
	Within Groups	311297.885	17	18311.640		
	Total	814511.505	22			
Del 3,5-dOg	Between Groups	2059.833	4	514.958	5.099	.011
	Within Groups	1312.818	13	100.986		
	Total	3372.651	17			
Pel 3,5 -dOg	Between Groups	16529.132	3	5509.711	6.365	.013
	Within Groups	7790.504	9	865.612		
	Total	24319.636	12			
Cyn 3,5-O-glu	Between Groups	3156.433	5	631.287	29.300	.000
	Within Groups	366.275	17	21.546		
	Total	3522.707	22			

Table A.10: Phenolic and anthocyanin analysis for bioavailability samples.

Compound		Sum of Squares	df	Mean Square	F	Sig.
Gallic Acid-PG	Between Groups		5	1397805.246	6.782	.001
	Within Groups	3297633.779	16	206102.111		
	Total	1.029E7	21			
Gallic Acid-OUT	Between Groups	3465421.948	4	866355.487	27.053	.000
	Within Groups	416316.860	13	32024.374		
	Total	3881738.809	17			
Catechin-PG	Between Groups	9.766E8	4	2.442E8	4.777	.045
	Within Groups	3.067E8	6	5.112E7		
	Total	1.283E9	10			
Ferulic Acid-PG	Between Groups	325544.864	5	65108.973	52.077	.000
	Within Groups	20003.863	16	1250.241		
	Total	345548.726	21			
Ferulic Acid-IN	Between Groups	12106.775	5	2421.355	15.692	.000
	Within Groups	2314.641	15	154.309		
	Total	14421.416	20			
Ferulic Acid-OUT	Between Groups	309079.999	5	61816.000	22.980	.000
	Within Groups	43039.550	16	2689.972		
	Total	352119.548	21			
Q-3-g-PG	Between Groups	582669.542	5	116533.908	49.315	.000
	Within Groups	28356.341	12	2363.028		
	Total	611025.884	17			
Q-3-g-IN	Between Groups	12564.437	4	3141.109	10.039	.001
	Within Groups	3441.963	11	312.906		
	Total	16006.401	15			
Q-3-g-OUT	Between Groups	467196.996	4	116799.249	21.809	.000
	Within Groups	48200.976	9	5355.664		
	Total	515397.972	13			
Q-3-BDg-PG	Between Groups	7820116.383	5	1564023.277	11.847	.000
	Within Groups	1848185.238	14	132013.231		
	Total	9668301.621	19			
Q-3-BDg-IN	Between Groups	293231.614	5	58646.323	7.251	.001
	Within Groups	121320.223	15	8088.015		
	Total	414551.837	20			
Q-3-BDg-OUT	Between Groups	2,861E7	5	5721382,725	37,767	,000
	Within Groups	2423839,737	16	151489,984		
	Total	3,103E7	21			
Cyn 3-O-glu	Between Groups	34648.783	5	6929.757	20.938	.000
	Within Groups	4633.561	14	330.969		
	Total	39282.344	19			
Del 3,5-dOg	Between Groups	14054.325	4	3513.581	3.196	.046
	Within Groups	15389.842	14	1099.274		
	Total	29444.167	18			
Pel 3,5-dOg	Between Groups	333177.926	4	83294.482	14.388	.000
	Within Groups	75258.181	13	5789.091		
	Total	408436.108	17			
Pel 3-O-glu	Between Groups	5562.724	4	1390.681	70.457	.000
	Within Groups	236.856	12	19.738		
	Total	5799.580	16			

Table A.11: Regression analysis for total phenolic contents.

Methods		Sum of Squares	df	Mean Square	F	Sig.
CUPRAC	Regression	7.075E9	1	7.075E9	193.198	.000
	Residual	4.761E8	13	3.662E7		
	Total	7.551E9	14			
DPPH	Regression	1.507E9	1	1.507E9	451.962	.000
	Residual	4.334E7	13	3334172.316		
	Total	1.550E9	14			
FRAP	Regression	5.173E8	1	5.173E8	473.169	.000
	Residual	1.421E7	13	1093288.615		
	Total	5.315E8	14			
ABTS	Regression	2.242E9	1	2.242E9	263.105	.000
	Residual	1.108E8	13	8522484.463		
	Total	2.353E9	14			

Table A.12: Regression analysis for total flavonoid contents.

Methods		Sum of Squares	df	Mean Square	F	Sig.
CUPRAC	Regression	7.136E9	1	7.136E9	223.427	.000
	Residual	4.152E8	13	3.194E7		
	Total	7.551E9	14			
DPPH	Regression	1.534E9	1	1.534E9	1.256E3	.000
	Residual	1.588E7	13	1221909.347		
	Total	1.550E9	14			
FRAP	Regression	5.222E8	1	5.222E8	729.906	.000
	Residual	9301050.820	13	715465.448		
	Total	5.315E8	14			
ABTS	Regression	2.313E9	1	2.313E9	752.553	.000
	Residual	3.996E7	13	3073726.791		
	Total	2.353E9	14			

Table A.13: Regression analysis for total anthocyanin contents.

Methods		Sum of Squares	df	Mean Square	F	Sig.
CUPRAC	Regression	1.525E7	1	1.525E7	.026	.874
	Residual	7.536E9	13	5.797E8		
	Total	7.551E9	14			
DPPH	Regression	622.089	1	622.089	.000	.998
	Residual	1.550E9	13	1.193E8		
	Total	1.550E9	14			
FRAP	Regression	385529.294	1	385529.294	.009	.924
	Residual	5.311E8	13	4.086E7		
	Total	5.315E8	14			
ABTS	Regression	2549014.614	1	2549014.614	.014	.907
	Residual	2.351E9	13	1.808E8		
	Total	2.353E9	14			

Table A.14: Regression analysis for total tannin contents.

Methods		Sum of Squares	df	Mean Square	F	Sig.
CUPRAC	Regression	6.112E9	1	6.112E9	55.189	.000
	Residual	1.440E9	13	1.107E8		
	Total	7.551E9	14			
DPPH	Regression	1.176E9	1	1.176E9	40.819	.000
	Residual	3.745E8	13	2.880E7		
	Total	1.550E9	14			
FRAP	Regression	3.785E8	1	3.785E8	32.141	.000
	Residual	1.531E8	13	1.177E7		
	Total	5.315E8	14			
ABTS	Regression	1.675E9	1	1.675E9	32.111	.000
	Residual	6.781E8	13	5.216E7		
	Total	2.353E9	14			

Table A.15: Regression analysis for total antioxidant activity by CUPRAC method.

Methods		Sum of Squares	df	Mean Square	F	Sig.
DPPH	Regression	1.506E9	1	1.506E9	439.996	.000
	Residual	4.449E7	13	3422248.383		
	Total	1.550E9	14			
FRAP	Regression	5.005E8	1	5.005E8	209.674	.000
	Residual	3.103E7	13	2387001.710		
	Total	5.315E8	14			
ABTS	Regression	2.186E9	1	2.186E9	169.676	.000
	Residual	1.675E8	13	1.288E7		
	Total	2.353E9	14			

Table A.16: Regression analysis for total antioxidant activity by DPPH method.

Methods		Sum of Squares	df	Mean Square	F	Sig.
FRAP	Regression	5.248E8	1	5.248E8	1.014E3	.000
	Residual	6725255.933	13	517327.379		
	Total	5.315E8	14			
ABTS	Regression	2.308E9	1	2.308E9	660.651	.000
	Residual	4.541E7	13	3493059.594		
	Total	2.353E9	14			

Table A.17: Regression analysis for total antioxidant activity by FRAP method.

Methods		Sum of Squares	df	Mean Square	F	Sig.
ABTS	Regression	2.308E9	1	2.308E9	671.041	.000
	Residual	4.472E7	13	3440000.180		
	Total	2.353E9	14			

APPENDIX B

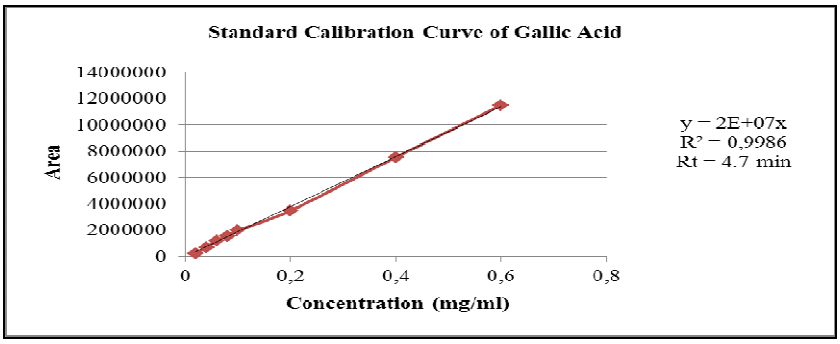

Figure B.1: Standard calibration curve of gallic acid for HPLC.

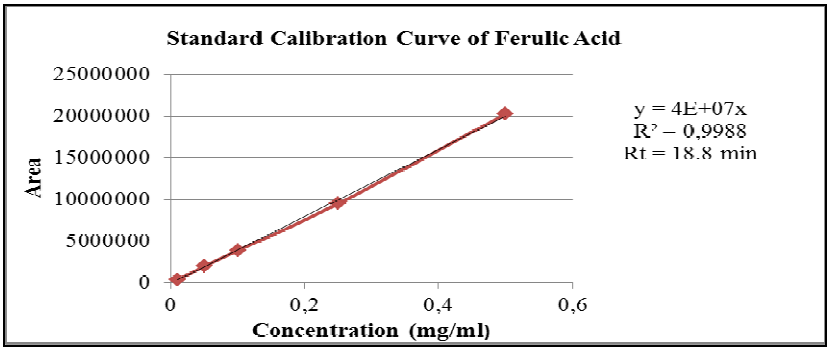

Figure B.2: Standard calibration curve of ferulic acid for HPLC.

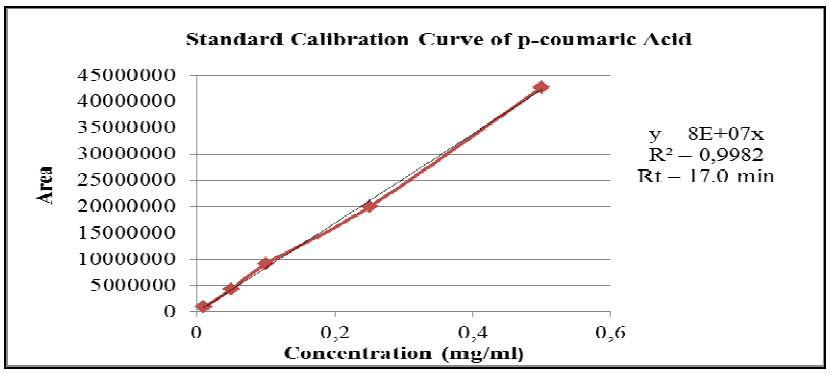

Figure B.3: Standard calibration curve of p-coumaric acid for HPLC.

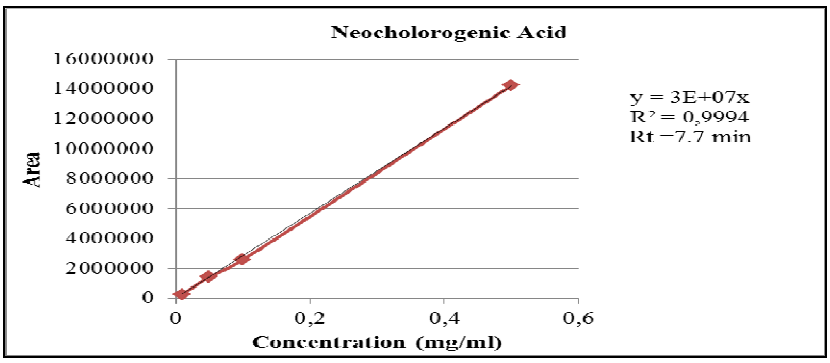

Figure B.4: Standard calibration curve of neochlorogenic acid for HPLC.

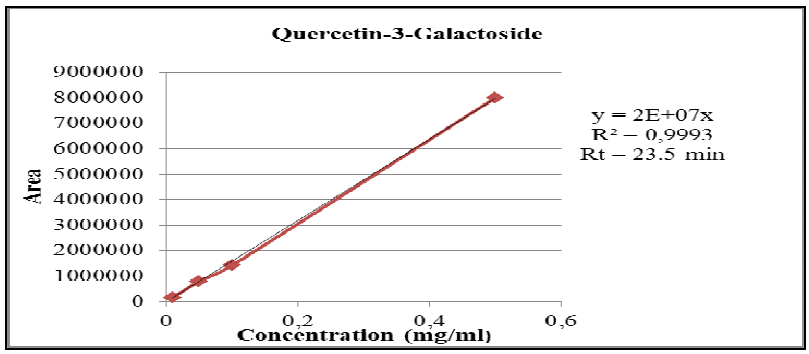

Figure B.5: Standard calibration curve of q-3-g for HPLC.

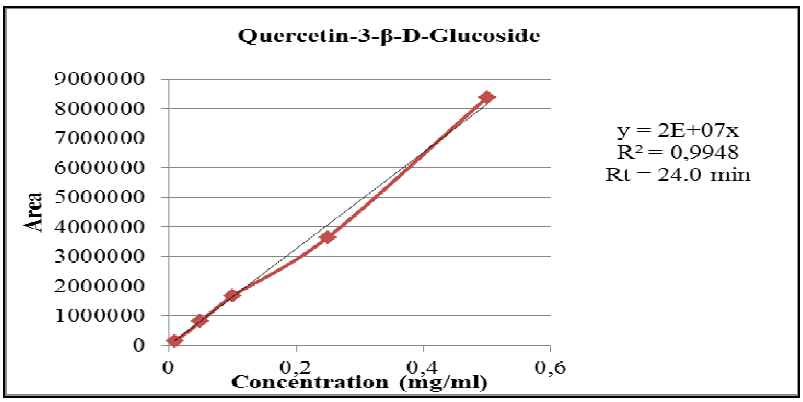

Figure B.6: Standard calibration curve of q-3-BDg for HPLC.

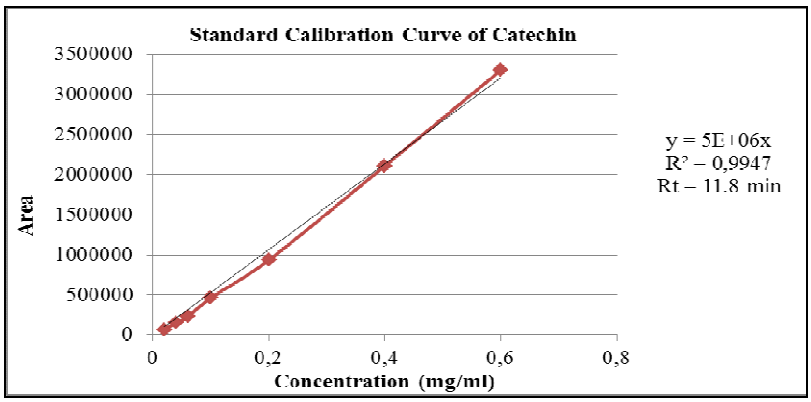

Figure B.7: Standard calibration curve of catechin for HPLC.

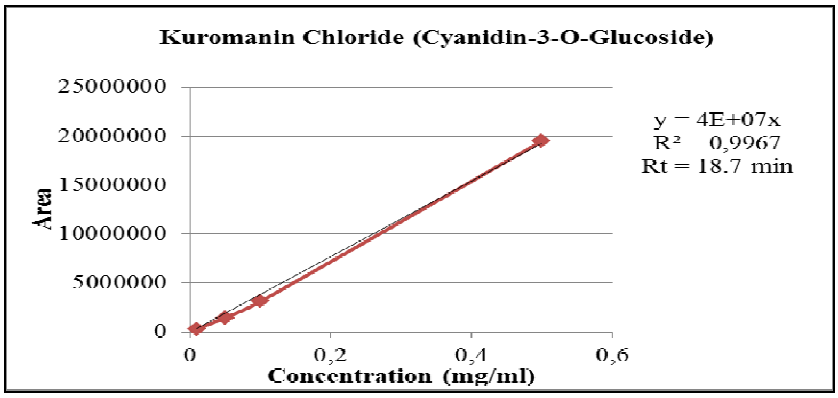

Figure B.8: Standard calibration curve of cyn 3-O-glu for HPLC.

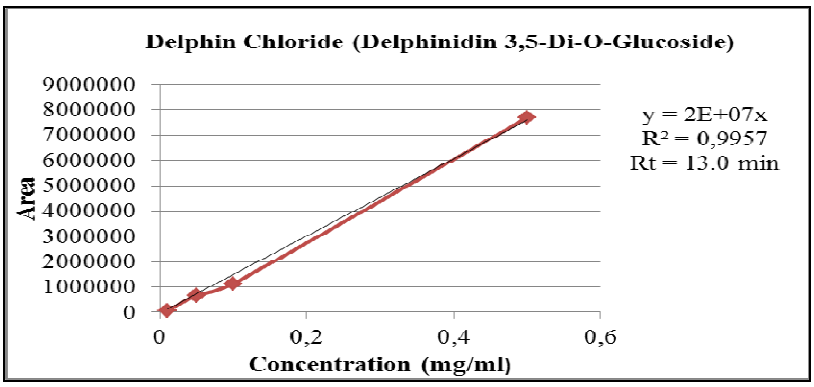

Figure B.9: Standard calibration curve of del 3,5-dOg for HPLC.

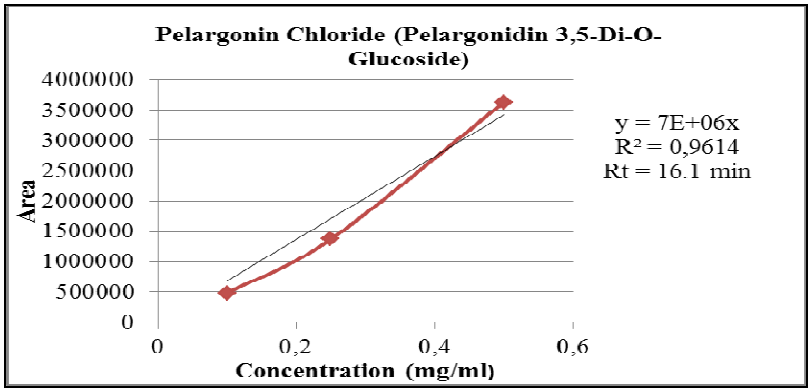

Figure B.10: Standard calibration curve of pel 3,5-dOg for HPLC.

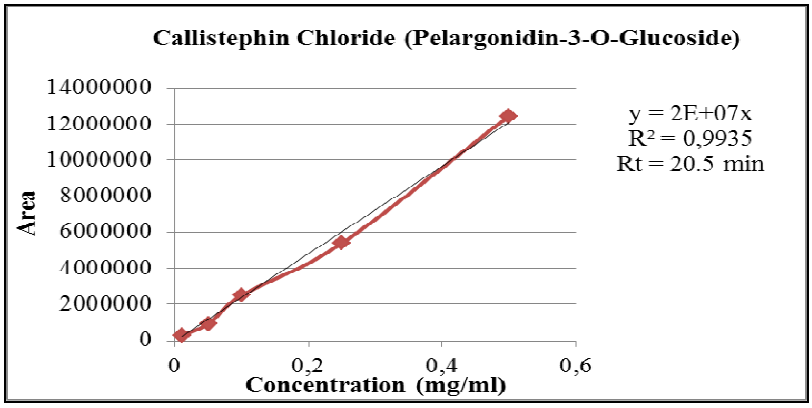

Figure B.11: Standard calibration curve of pel 3-O-glu for HPLC.

APPENDIX C

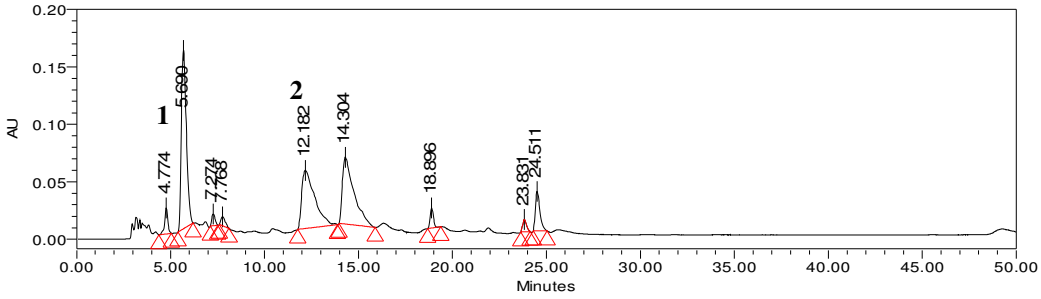

Figure C.1: Representative HPLC chromatogram of peel at 280 nm.

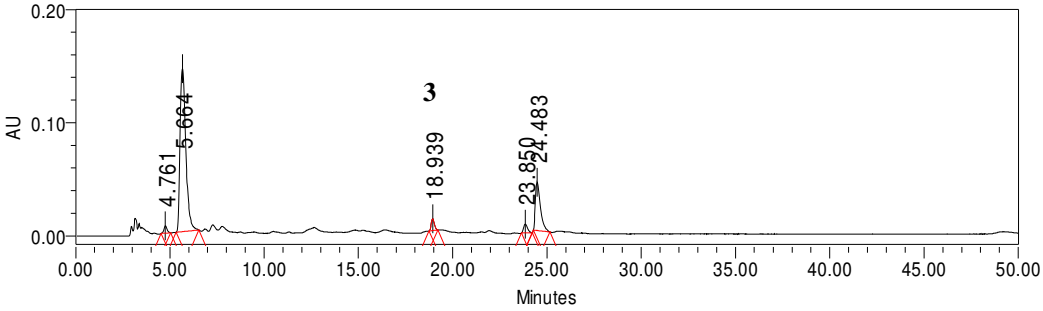

Figure C.2: Representative HPLC chromatogram of peel at 312 nm.

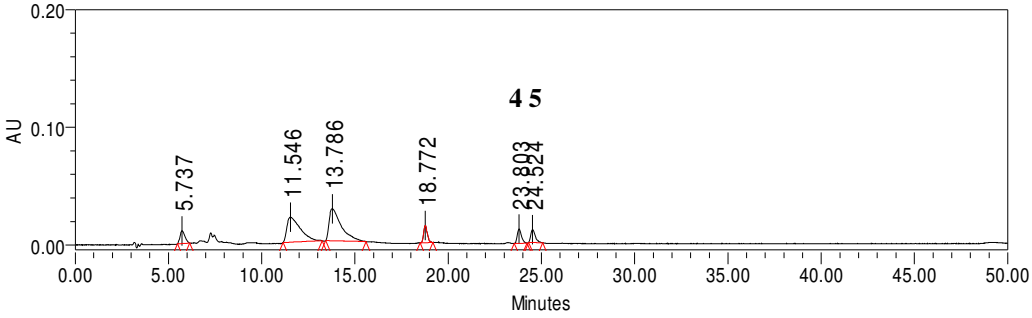

Figure C.3: Representative HPLC chromatogram of peel at 360 nm.

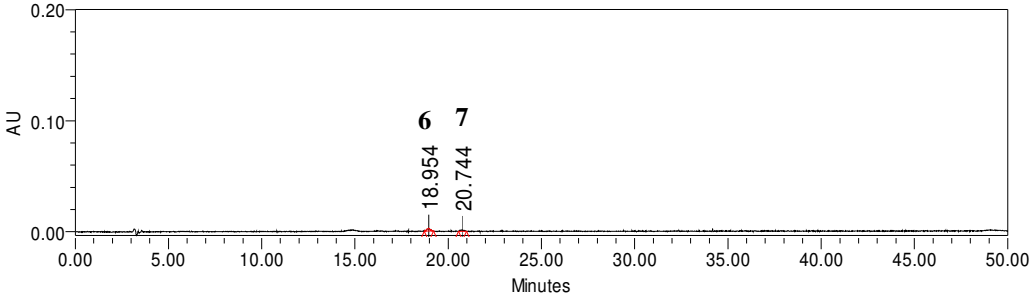

Figure C.4: Representative HPLC chromatogram of peel at 520 nm.

(1: gallic acid, 2: catechin, 3: ferulic acid, 4: p-coumaric acid, 4: q-3-g, 5: q-3-BDg, 6: cyn 3-O-glu, 7: pel 3-O-glu)

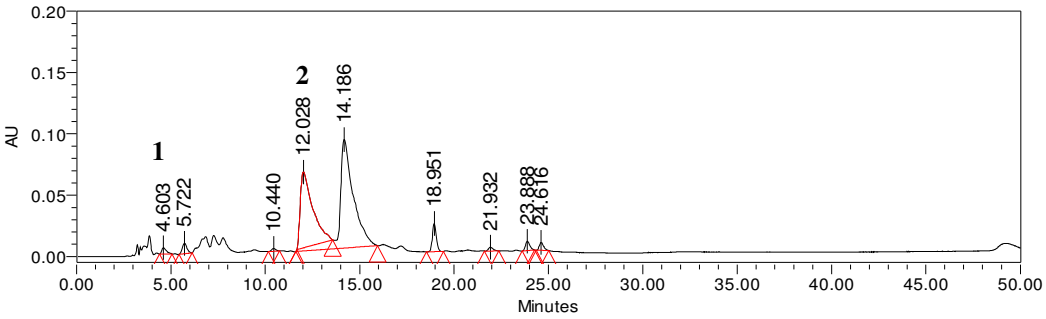

Figure C.5: Representative HPLC chromatogram of raw material at 280 nm.

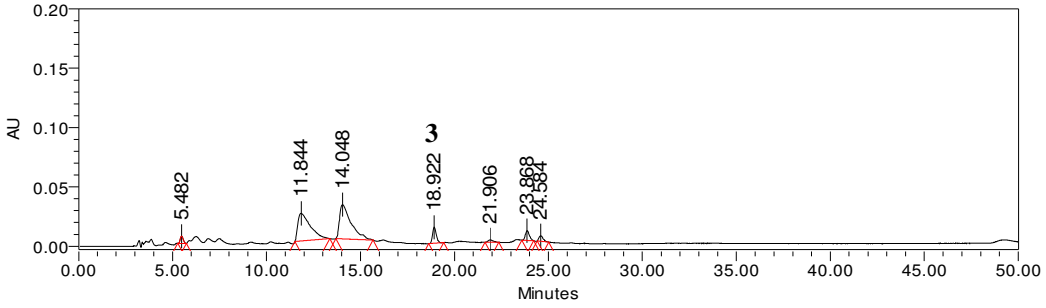

Figure C.6: Representative HPLC chromatogram of raw material at 312 nm.

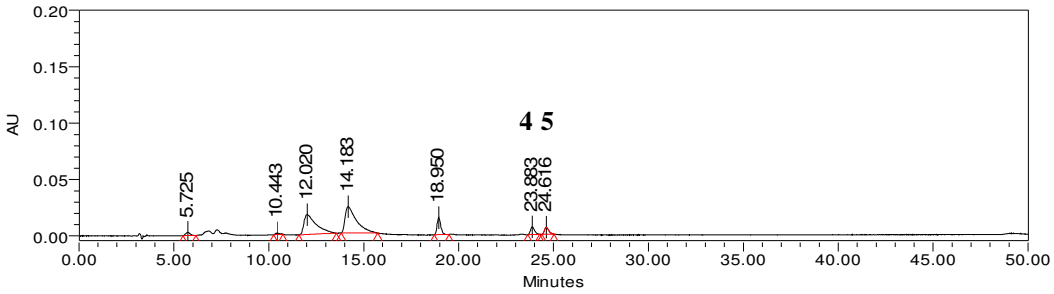

Figure C.7: Representative HPLC chromatogram of raw material at 360 nm.

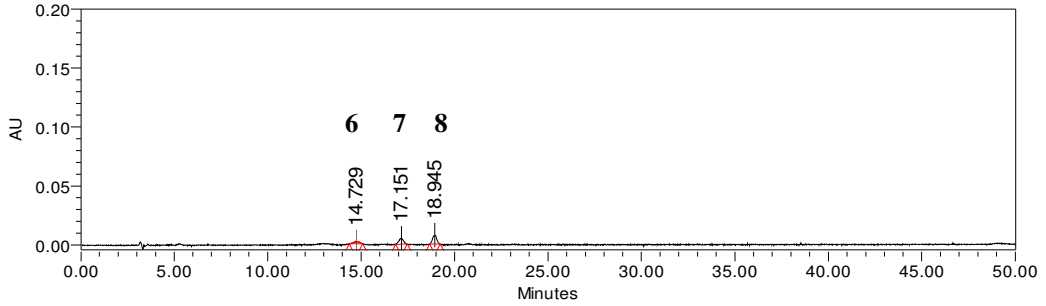

Figure C.8: Representative HPLC chromatogram of raw material at 520 nm.

(1: gallic acid, 2: catechin, 3: ferulic acid, 4: q-3-g, 5: q-3-BDg, 6: del 3,5-dOg, 7: pel 3,5-dOg, 8: cyn 3-O-glu)

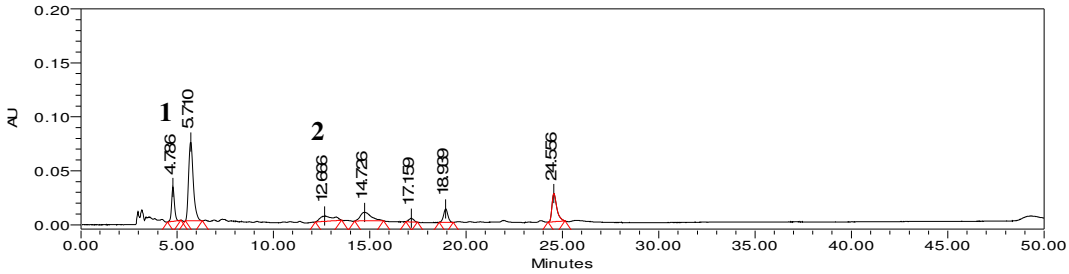

Figure C.9: Representative HPLC chromatogram of arils at 280 nm.

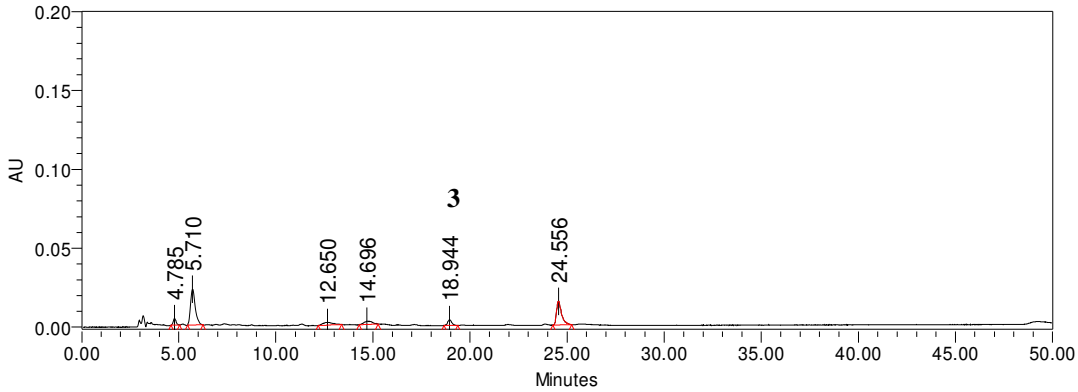

Figure C.10: Representative HPLC chromatogram of arils at 312 nm.

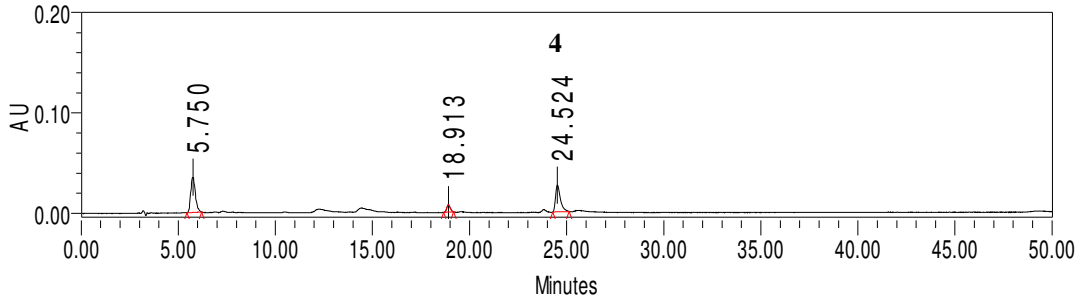

Figure C.11: Representative HPLC chromatogram of arils at 360 nm.

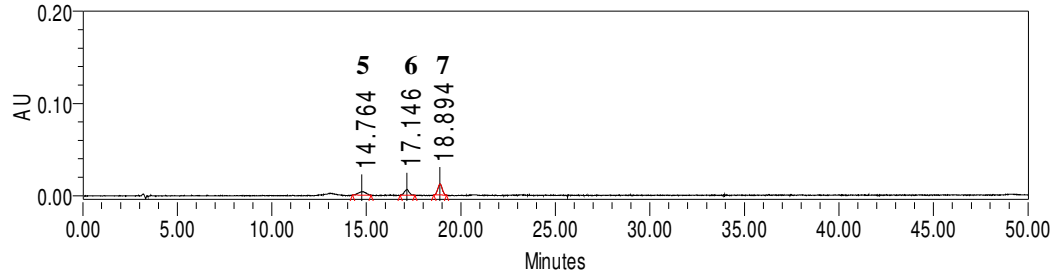

Figure C.12: Representative HPLC chromatogram of arils at 520 nm.

(1: gallic acid, 2: catechin, 3: ferulic acid, 4: q-3-BDg, 5: del 3,5-dOg, 6: pel 3,5-dOg, 7: cyn 3-O-glu)

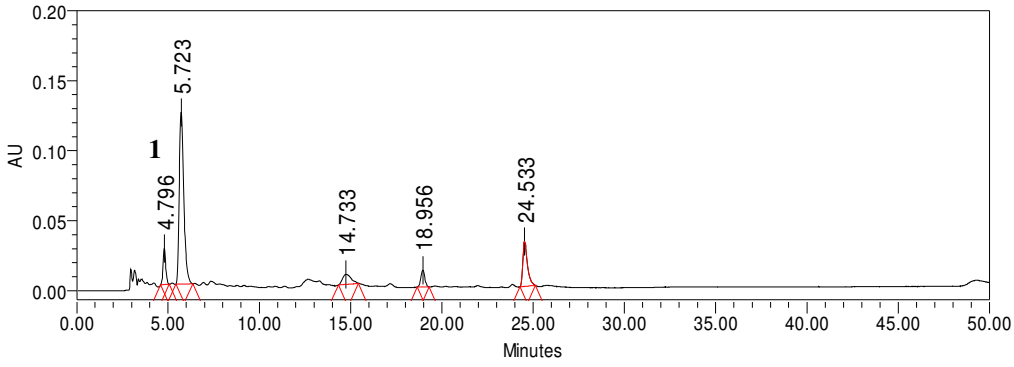

Figure C.13: Representative HPLC chromatogram of mashing at 280 nm.

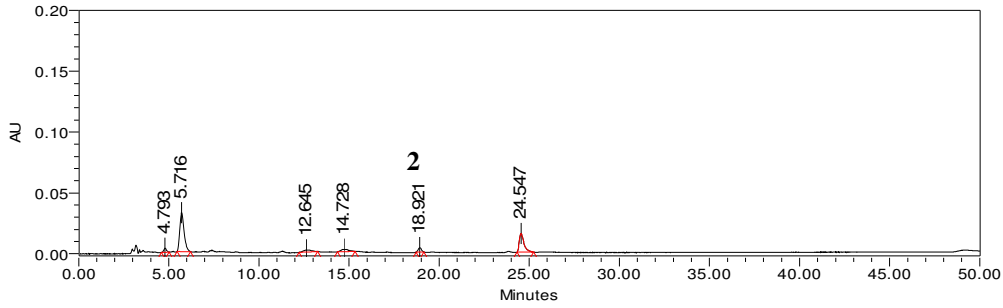

Figure C.14: Representative HPLC chromatogram of mashing at 312 nm.

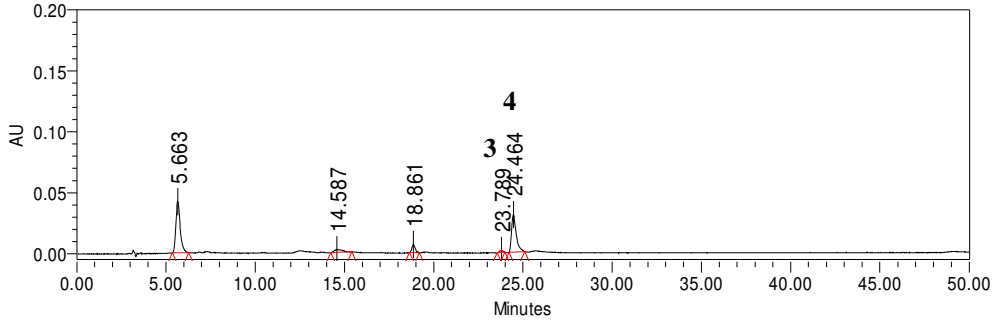

Figure C.15: Representative HPLC chromatogram of mashing at 360 nm.

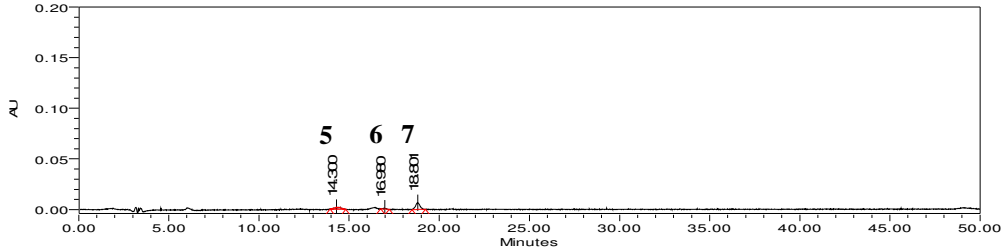

Figure C.16: Representative HPLC chromatogram of mashing at 520 nm.

(1: gallic acid, 2: ferulic acid, 3: q-3-g, 4: q-3-BDg, 5: del 3,5-dOg, 6: pel 3,5-dOg, 7: cyn 3-O-glu)

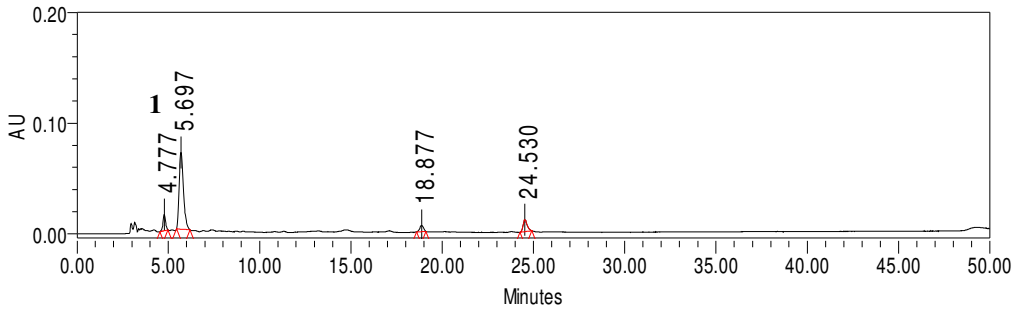

Figure C.17: Representative HPLC chromatogram of pressing at 280 nm.

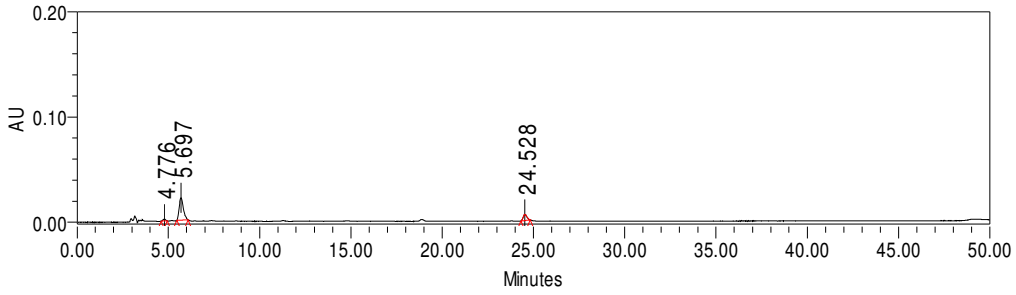

Figure C.18: Representative HPLC chromatogram of pressing at 312 nm.

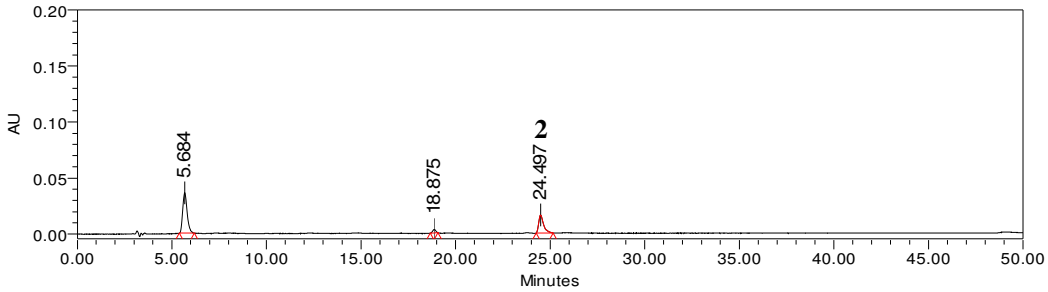

Figure C.19: Representative HPLC chromatogram of pressing at 360 nm.

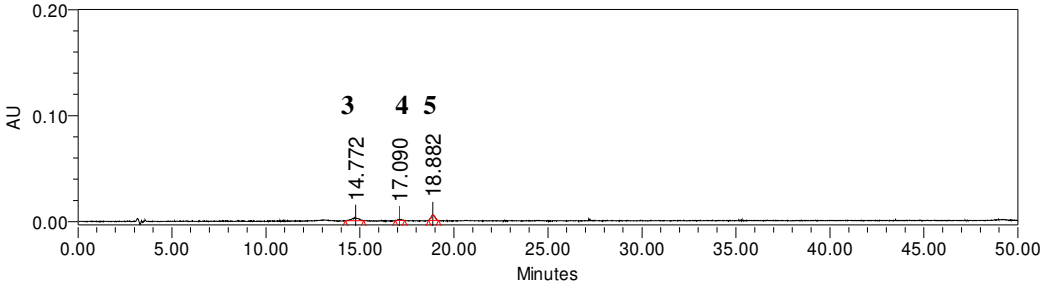

Figure C.20: Representative HPLC chromatogram of pressing at 520 nm.

(1: gallic acid, 2: q-3-BDg, 3: del 3,5-dOg, 4: pel 3,5-dOg, 5: cyn 3-O-glu)

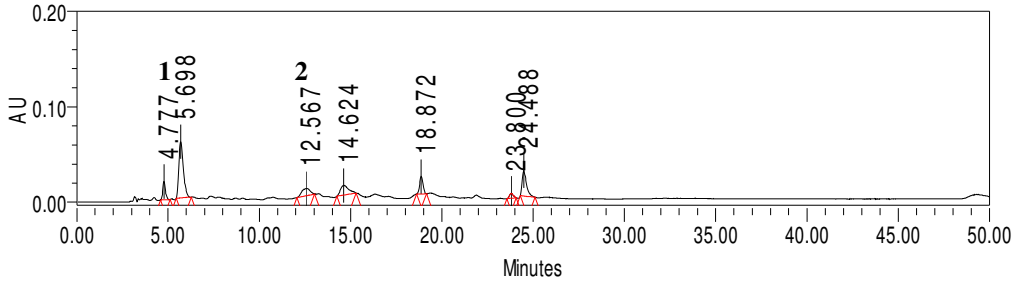

Figure C.21: Representative HPLC chromatogram of press cake at 280 nm.

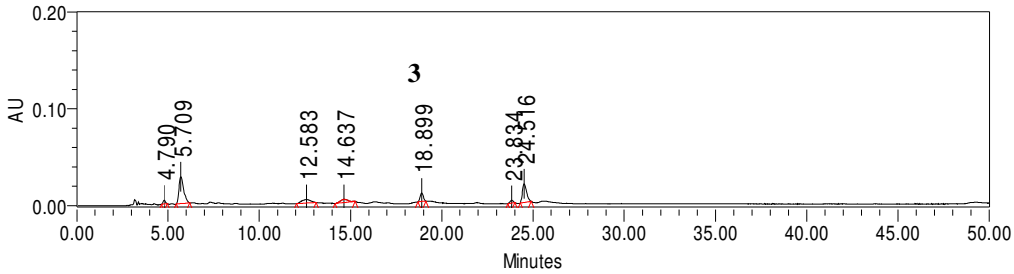

Figure C.22: Representative HPLC chromatogram of press cake at 312 nm.

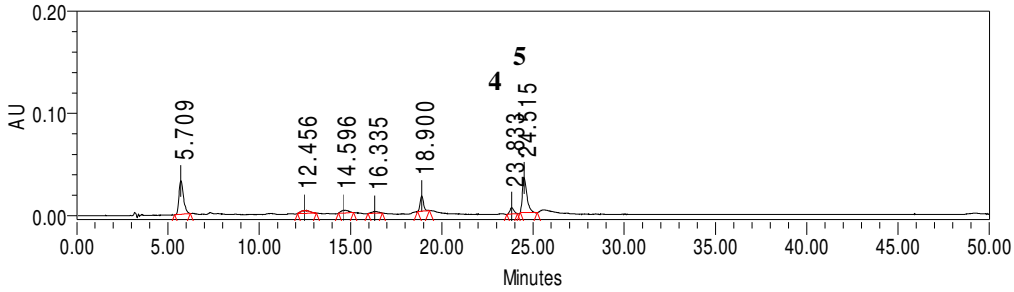

Figure C.23: Representative HPLC chromatogram of press cake at 360 nm.

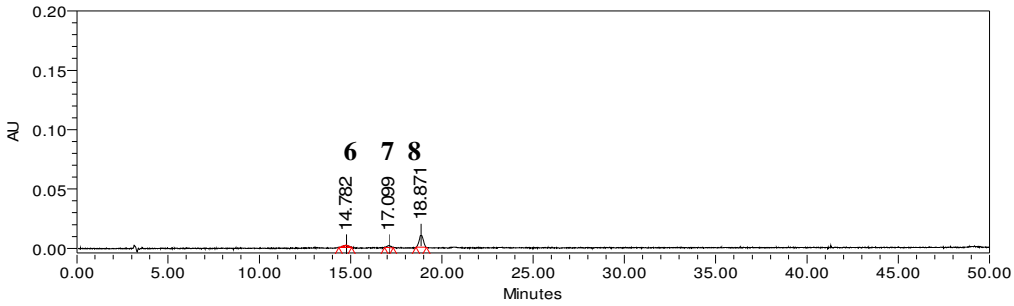

Figure C.24: Representative HPLC chromatogram of press cake at 520 nm.

(1: gallic acid, 2: catechin, 3: ferulic acid, 4: q-3-g, 5: q-3-BDg, 6: del 3,5-dOg, 7: pel 3,5-dOg, 8: cyn 3-O-glu)

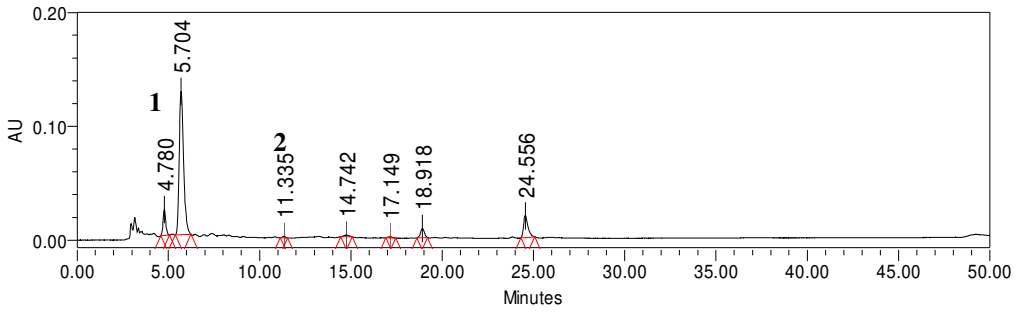

Figure C.25: Representative HPLC chromatogram of cooling at 280 nm.

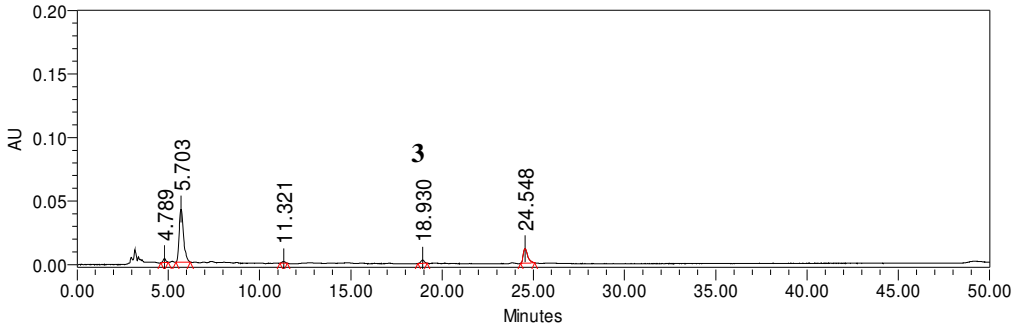

Figure C.26: Representative HPLC chromatogram of cooling at 312 nm.

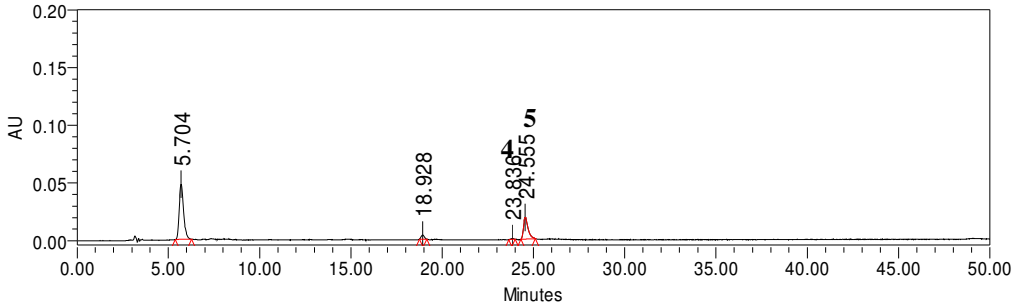

Figure C.27: Representative HPLC chromatogram of cooling at 520 nm.

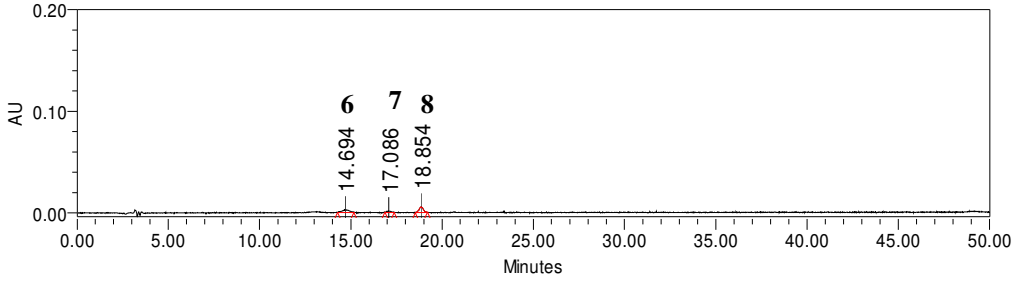

Figure C.28: Representative HPLC chromatogram of cooling at 520 nm.

(1: gallic acid, 2: catechin, 3: ferulic acid, 4: q-3-g, 5: q-3-BDg, 6: del 3,5-dOg, 7: pel 3,5-dOg, 8: cyn 3-O-glu)

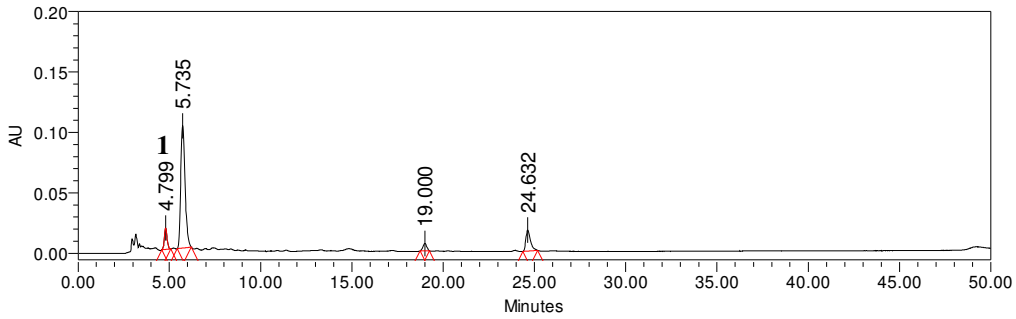

Figure C.29: Representative HPLC chromatogram of pasteurization at 280 nm.

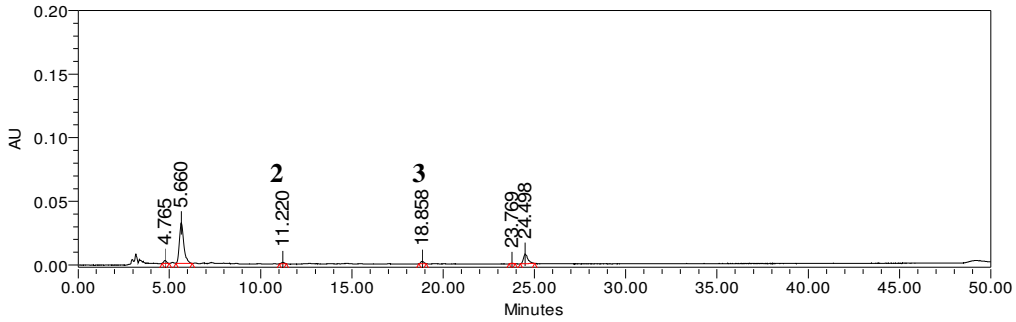

Figure C.30: Representative HPLC chromatogram of pasteurization at 312 nm.

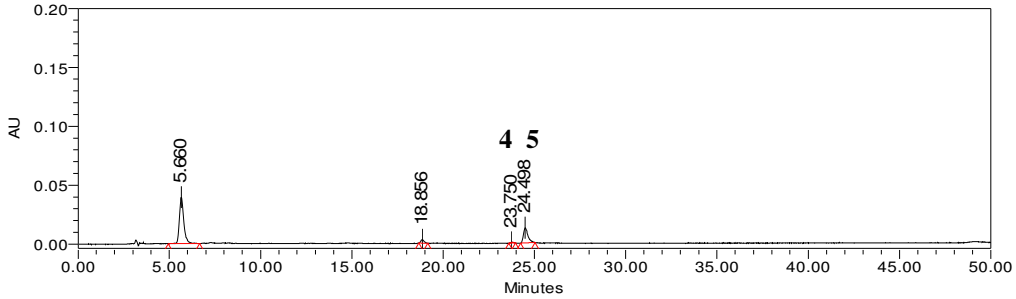

Figure C.31: Representative HPLC chromatogram of pasteurization at 360 nm.

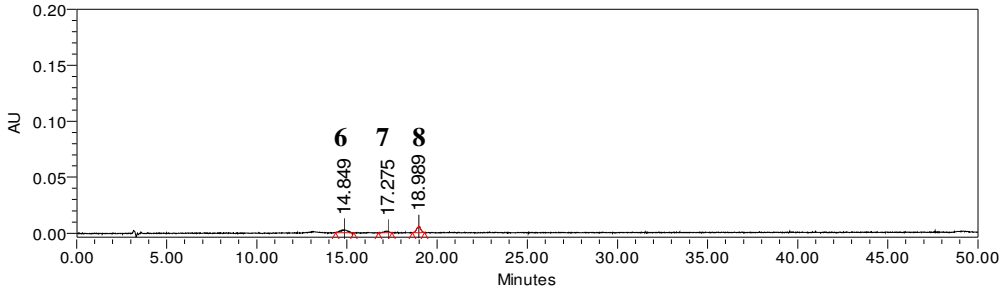

Figure C.32: Representative HPLC chromatogram of pasteurization at 520 nm.

(1: gallic acid, 2: chlorogenic acid, 3: ferulic acid, 4: q-3-g, 5: q-3-BDg, 6: del 3,5-dOg, 7: pel 3,5-dOg, 8: cyn 3-O-glu)

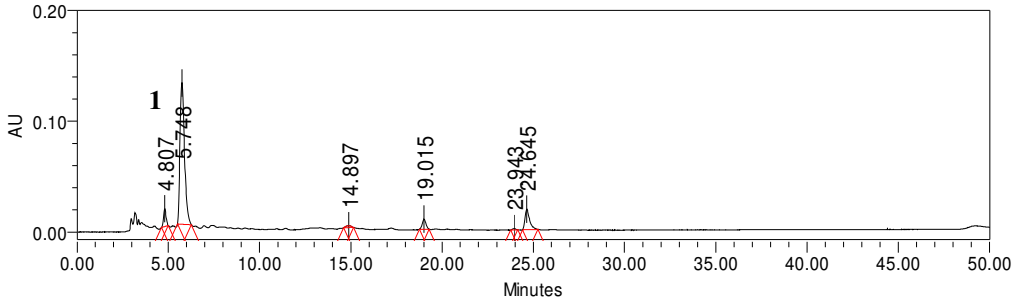

Figure C.33: Representative HPLC chromatogram of enzyme application at 280 nm.

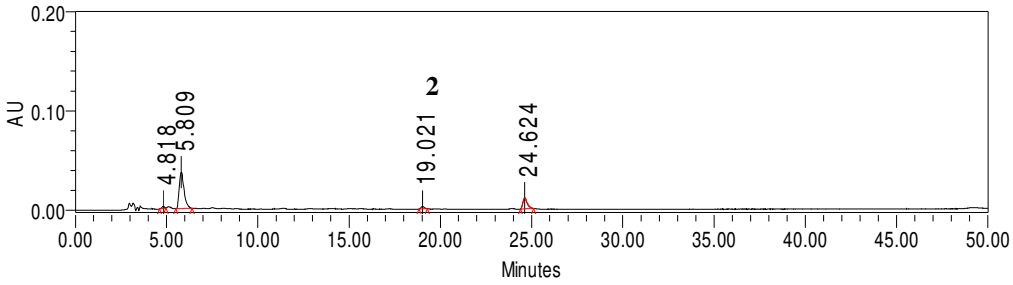

Figure C.34: Representative HPLC chromatogram of enzyme application at 312 nm.

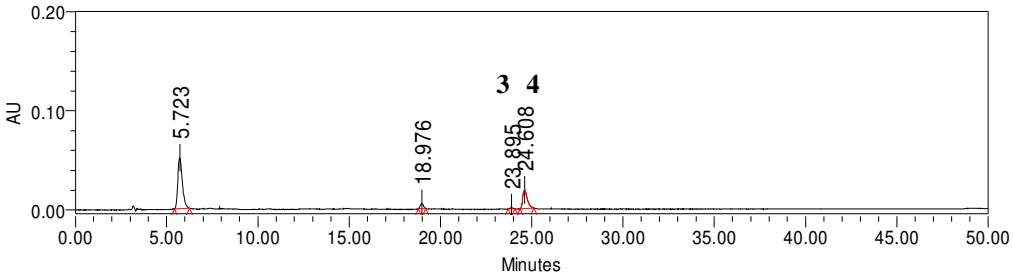

Figure C.35: Representative HPLC chromatogram of enzyme application at 360 nm.

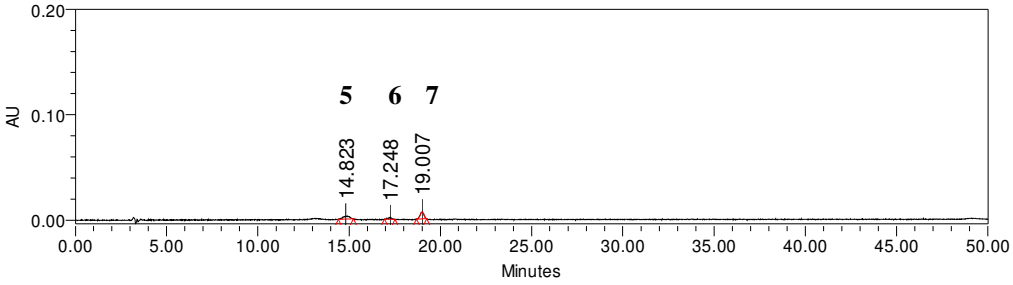

Figure C.36: Representative HPLC chromatogram of enzyme application at 520 nm.

(1: gallic acid, 2: ferulic acid, 3: q-3-g, 4: q-3-BDg, 5: del 3,5-dOg, 6: pel 3,5-dOg, 7: cyn 3-O-glu)

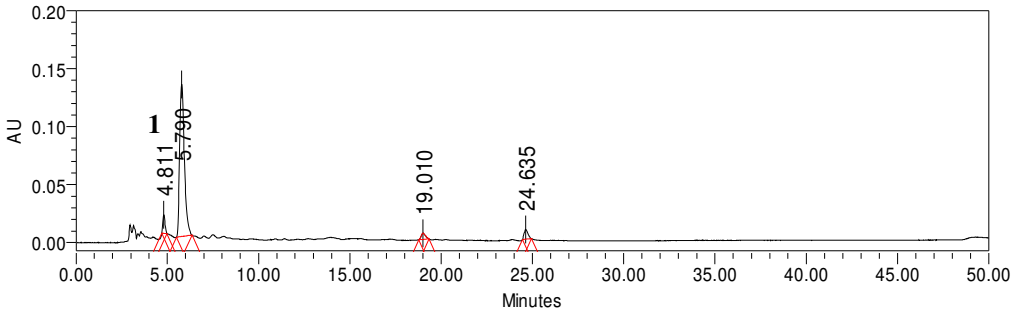

Figure C.37: Representative HPLC chromatogram of clarification at 280 nm.

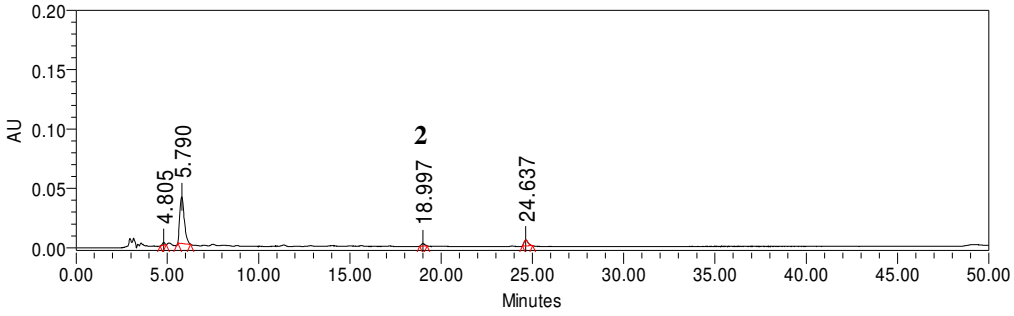

Figure C.38: Representative HPLC chromatogram of clarification at 312 nm.

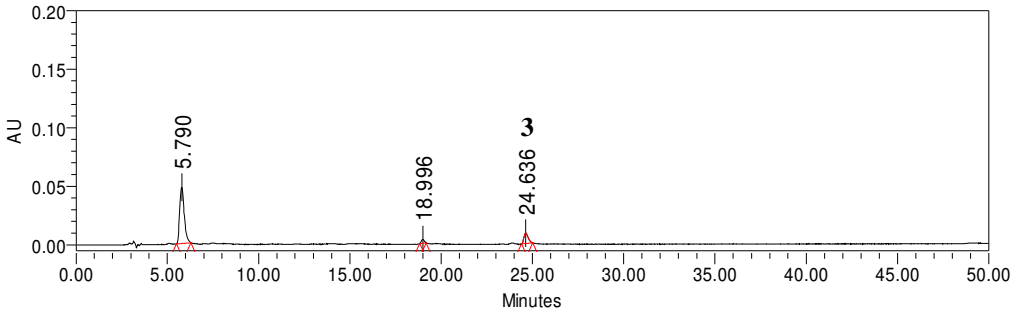

Figure C.39: Representative HPLC chromatogram of clarification at 360 nm.

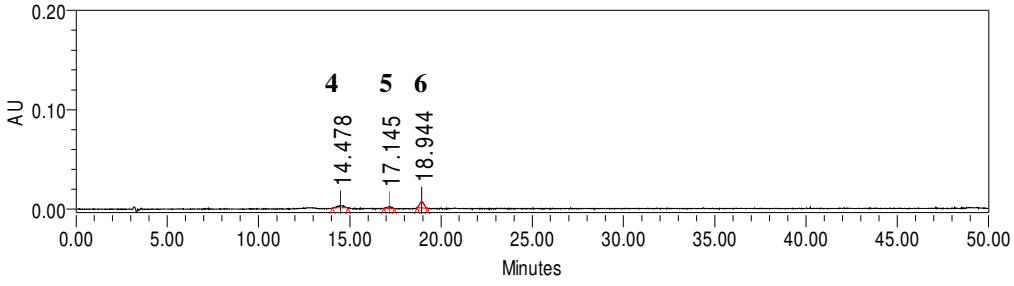

Figure C.40: Representative HPLC chromatogram of clarification at 520 nm.

(1: gallic acid, 2: ferulic acid, 3: q-3-BDg, 4: del 3,5-dOg, 5: pel 3,5-dOg, 6: cyn 3-O-glu)

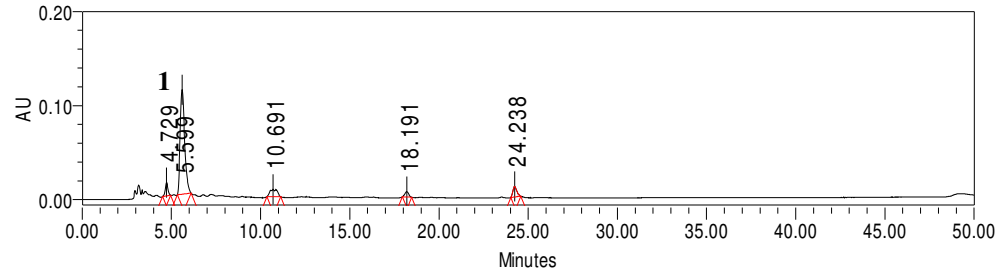

Figure C.41: Representative HPLC chromatogram of precipitate at 280 nm.

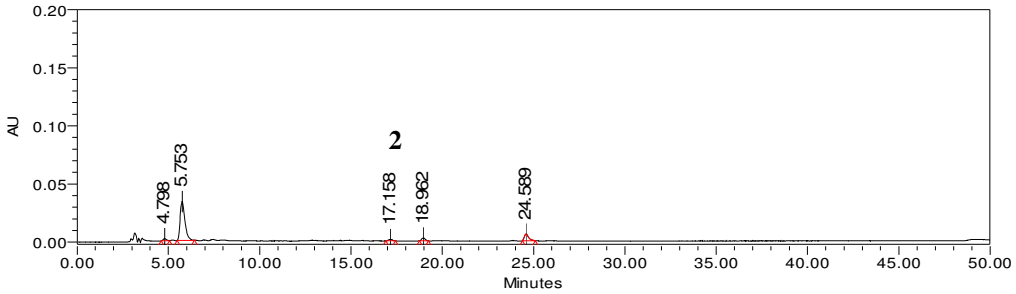

Figure C.42: Representative HPLC chromatogram of precipitate at 312 nm.

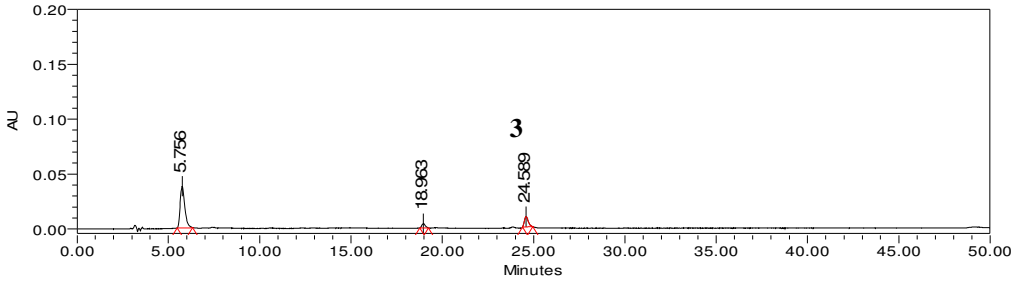

Figure C.43: Representative HPLC chromatogram of precipitate at 360 nm.

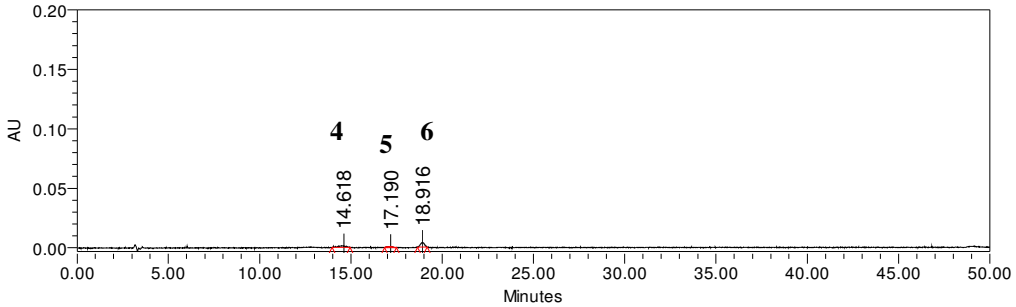

Figure C.44: Representative HPLC chromatogram of precipitate at 520 nm.

(1: gallic acid, 2: ferulic acid, 3: q-3-BDg, 4: del 3,5-dOg, 5: pel 3,5-dOg, 6: cyn 3-O-glu)

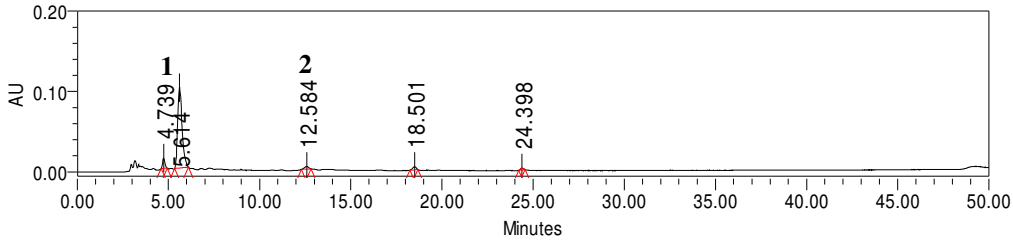

Figure C.45: Representative HPLC chromatogram of ultrafiltration at 280 nm.

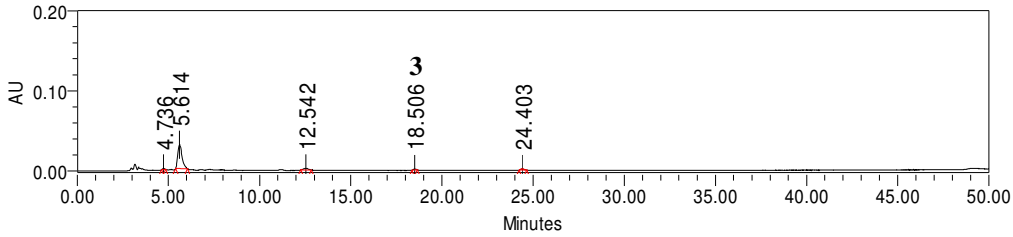

Figure C.46: Representative HPLC chromatogram of ultrafiltraion at 312 nm.

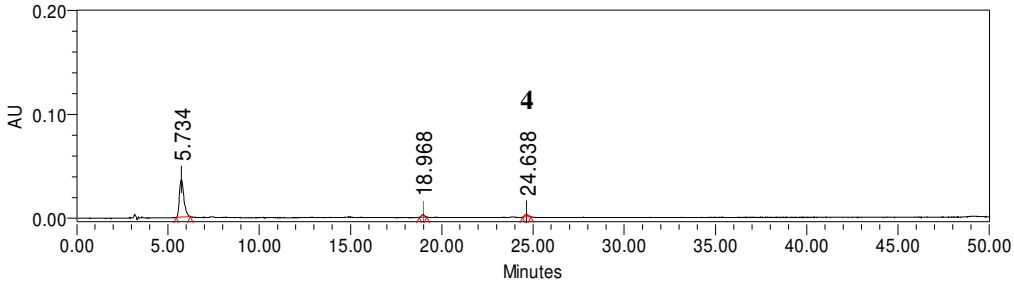

Figure C.47: Representative HPLC chromatogram of ultrafiltration at 360 nm.

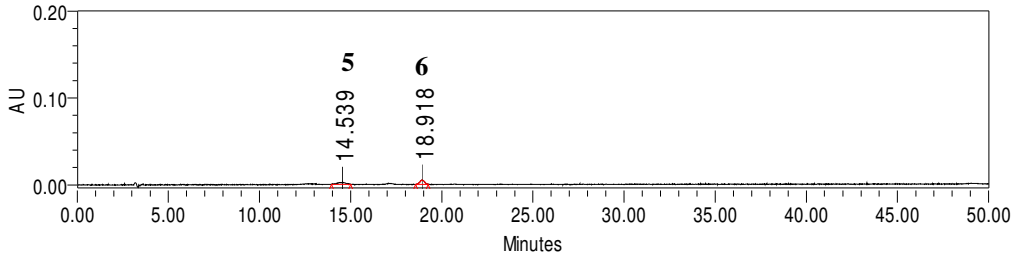

Figure C.48: Representative HPLC chromatogram of ultrafiltration at 520 nm.

(1: gallic acid, 2: catechin, 3: ferulic acid, 4: q-3-BDg, 5: del 3,5-dOg, 6: cyn 3-O-glu)

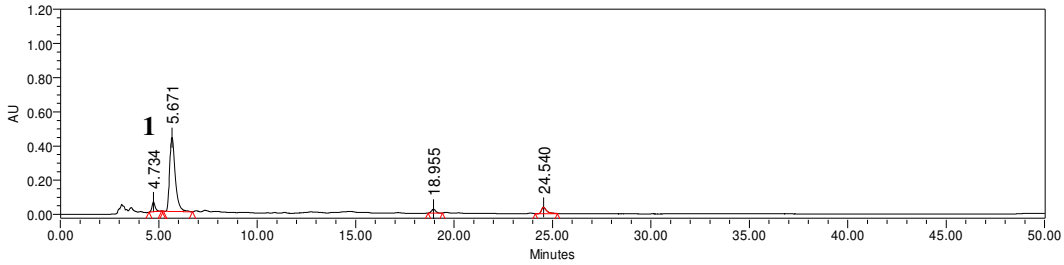

Figure C.49: Representative HPLC chromatogram of concentrate at 280 nm.

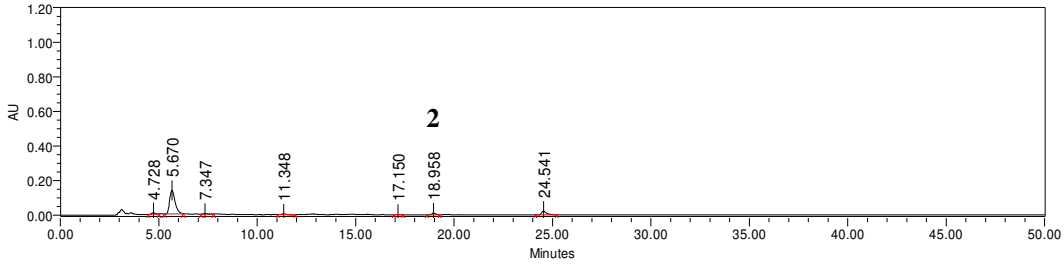

Figure C.50: Representative HPLC chromatogram of concentrate at 312 nm.

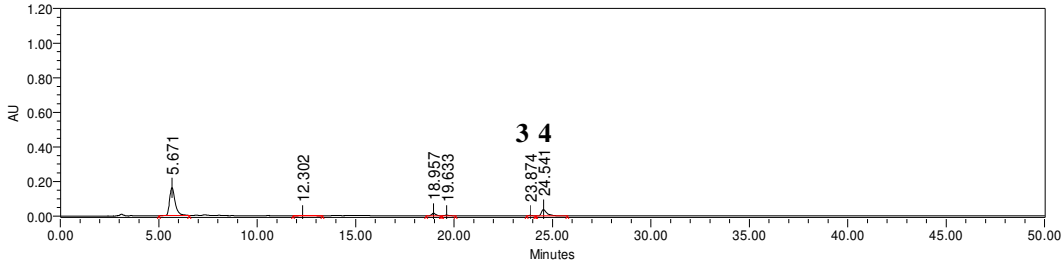

Figure C.51: Representative HPLC chromatogram of concentrate at 360 nm.

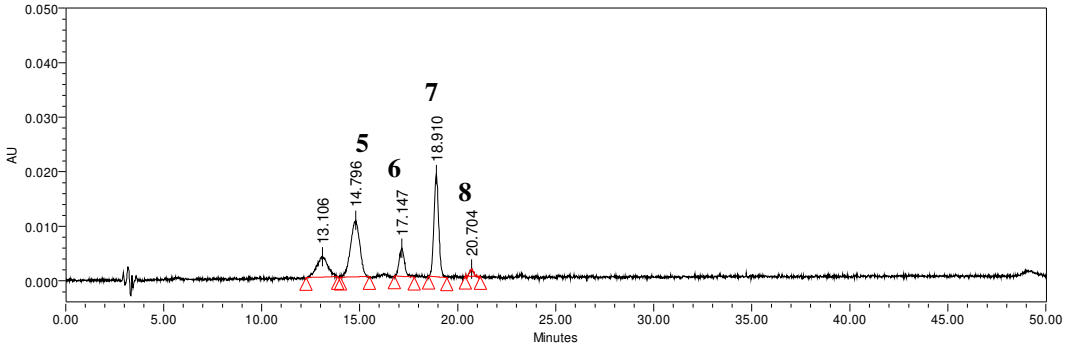

Figure C.52: Representative HPLC chromatogram of concentrate at 520 nm.

(1: gallic acid, 2: ferulic acid, 3: q-3-g, 4: q-3-BDg, 5: del 3,5-dOg, 6: pel 3,5-dOg, 7: cyn 3-O-glu, 8: pel 3-O-glu)

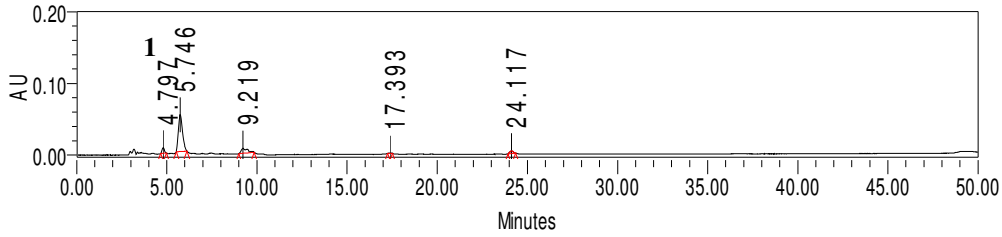

Figure C.53: Representative HPLC chromatogram of nectar at 280 nm.

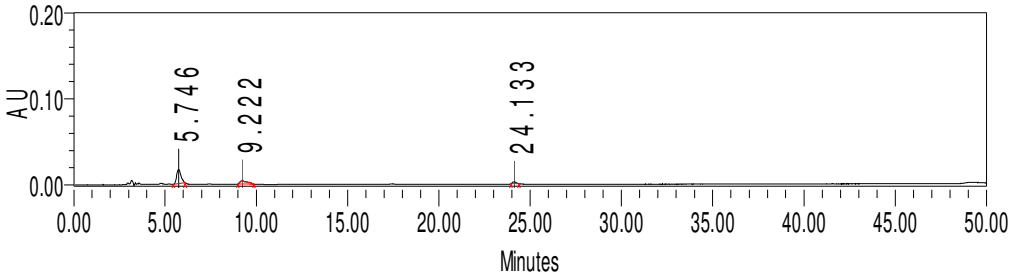

Figure C.54: Representative HPLC chromatogram of nectar at 312 nm.

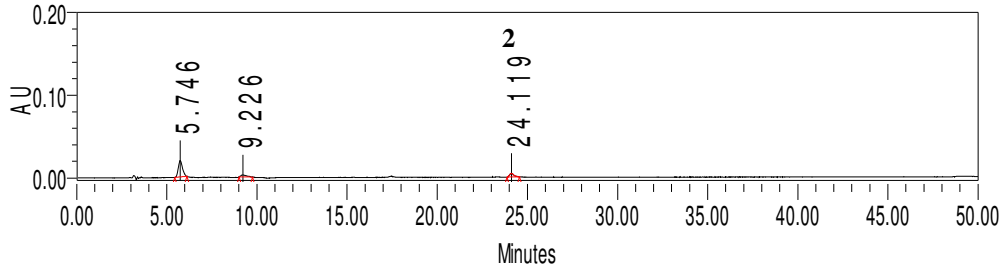

Figure C.55: Representative HPLC chromatogram of nectar at 360 nm.

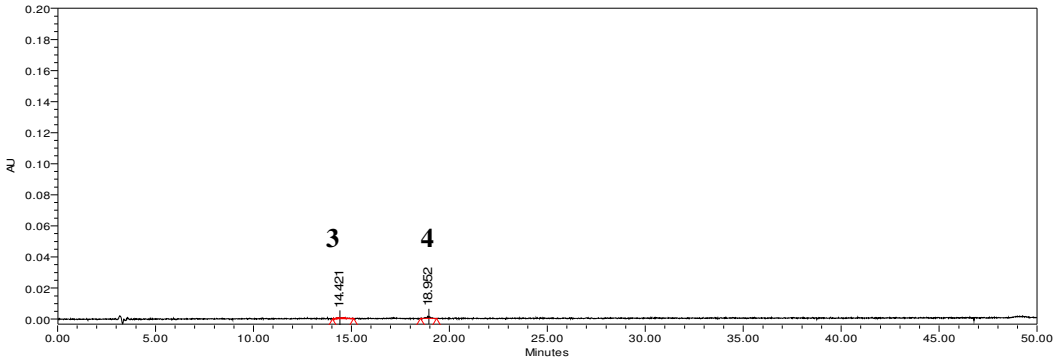

Figure C.56: Representative HPLC chromatogram of nectar at 520 nm.

(1: gallic acid, 2: q-3-BDg, 3: del 3,5-dOg, 4: cyn 3-O-glu)

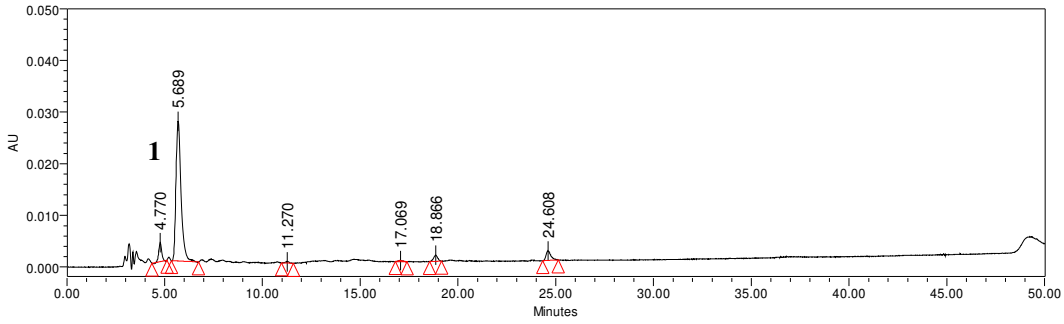

Figure C.57: Representative HPLC chromatogram of pasteurized nectar at 280 nm.

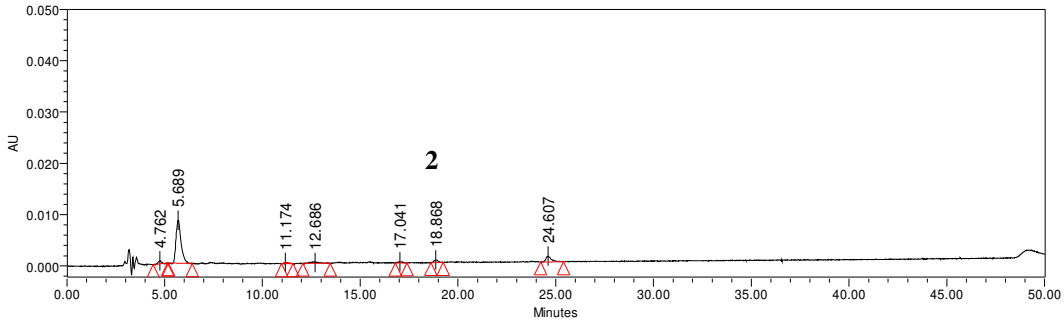

Figure C.58: Representative HPLC chromatogram of pasteurized nectar at 312 nm.

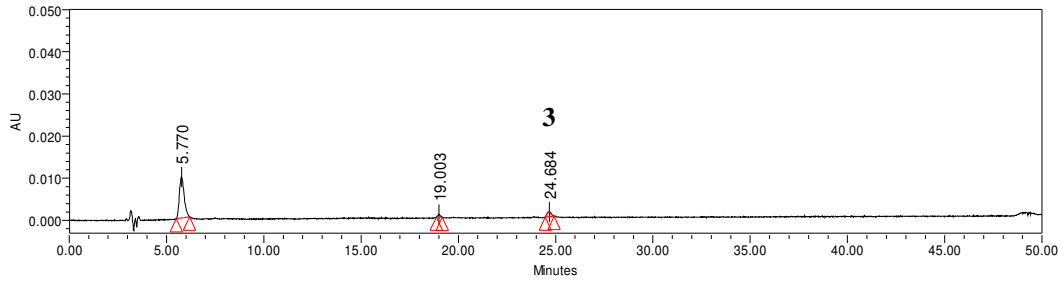

Figure C.59: Representative HPLC chromatogram of pasteurized nectar at 360 nm.

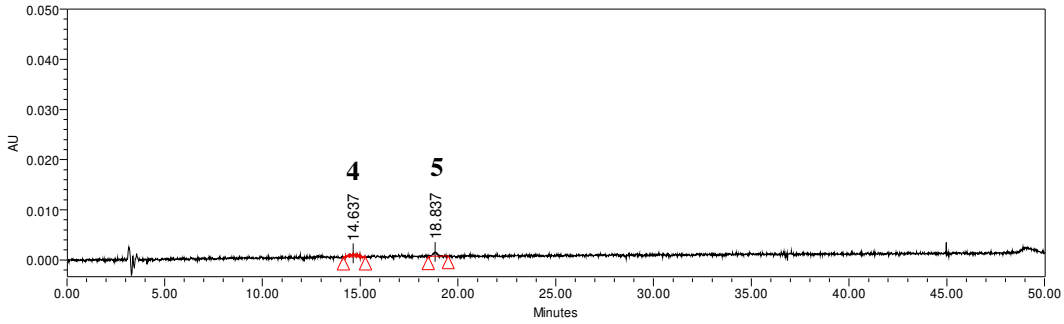

Figure C.60: Representative HPLC chromatogram of pasteurized nectar at 520 nm.

(1: gallic acid, 2: ferulic acid, 3: q-3-BDg, 4: del 3,5-dOg, 5: cyn 3-O-glu)

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PUBLICATIONS/PRESENTATIONS ON THE THESIS

- Capanoğlu Güven, E., Erdil Nilüfer, D., Kapcı, B., **Surek, E.,** Suzme, S., Boyacıoglu, D., 2012. Changes occurring in antioxidant properties during fruit juice processing. *Gıda Teknolojisi, 16(*2): 100-104.
- **Surek E.,** Erdil Nilüfer, D, 2012. Changes in polyphenols and antioxidant activity during the processing of pomegranate into nectar. *International Congress-1st North European Congress on Food,* April 22 – 24, 2012 Saint Petersburg, Russia. (Poster).
- **Surek E.,** Erdil Nilüfer, D, 2012. Polyphenols and antioxidant activities of waste products obtained from pomegranate nectar processing. *International Congress- Advanced Nonthermal Processing in Food Technology*, May 7-10, 2012, Kusadasi, Turkey. (Poster).